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 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09   CA/CAplus records now contain indexing from 1907 to the
                   present
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 NEWS  6  OCT 10   PCTFULL: Two new display fields added
 NEWS  7  OCT 21   BIOSIS file reloaded and enhanced
 NEWS  8  OCT 28   BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9  NOV 24   MSDS-CCOHS file reloaded
 NEWS 10  DEC 08   CABA reloaded with left truncation
 NEWS 11  DEC 08   IMS file names changed
 NEWS 12  DEC 09   Experimental property data collected by CAS now available
                   in REGISTRY
 NEWS 13  DEC 09   STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14  DEC 17   DGENE: Two new display fields added
 NEWS 15  DEC 18   BIOTECHNO no longer updated
 NEWS 16  DEC 19   CROPU no longer updated; subscriber discount no longer
                   available
 NEWS 17  DEC 22   Additional INPI reactions and pre-1907 documents added to CAS
                   databases
 NEWS 18  DEC 22   IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19  DEC 22   ABI-INFORM now available on STN
 NEWS 20  JAN 27   Source of Registration (SR) information in REGISTRY updated
                   and searchable
 NEWS 21  JAN 27   A new search aid, the Company Name Thesaurus, available in
                   CA/CAplus
 NEWS 22  FEB 05   German (DE) application and patent publication number format
                   changes
 NEWS 23  MAR 03   MEDLINE and LMEDLINE reloaded
 NEWS 24  MAR 03   MEDLINE file segment of TOXCENTER reloaded
 NEWS 25  MAR 03   FRANCEPAT now available on STN
 NEWS 26  MAR 29   Pharmaceutical Substances (PS) now available on STN
 NEWS 27  MAR 29   WPIFV now available on STN
 NEWS 28  MAR 29   No connect hour charges in WPIFV until May 1, 2004
 NEWS 29  MAR 29   New monthly current-awareness alert (SDI) frequency in RAPRA

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=> file .biotech caplus
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FULL ESTIMATED COST

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7 FILES IN THE FILE LIST

=> s polymerase enhancer
L1 38 POLYMERASE ENHANCER

=> s (polymerase and (enhanc? (4a) process?))
L2 625 (POLYMERASE AND (ENHANC? (4A) PROCESS?))

=> s l2 and dup rem
L3 0 L2 AND DUP REM

=> s l1 and replication
L4 1 L1 AND REPLICATION

=> s l2 and PCR
L5 136 L2 AND PCR

=> s l5 and (clon or sequenc? or mutagenesis)
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"HELP COMMANDS" at an arrow prompt (=>).

=> s (l5 and (clon? or sequenc? or mutagenesis))
L6 68 (L5 AND (CLON? OR SEQUENC? OR MUTAGENESIS))

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 43 DUP REM L6 (25 DUPLICATES REMOVED)

=> d ibib abs l7 1-10

L7 ANSWER 1 OF 43 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2004087284 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14973201
TITLE: A novel strategy to engineer DNA polymerases for **enhanced processivity** and improved performance in vitro.
AUTHOR: Wang Yan; Prosen Dennis E; Mei Li; Sullivan John C; Finney Michael; Vander Horn Peter B
CORPORATE SOURCE: Department of Research and Development, MJ Bioworks Inc., 7000 Shoreline Court, South San Francisco, CA 94080, USA.
SOURCE: Nucleic acids research, (2004) 32 (3) 1197-207.
Journal code: 0411011. ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20040224
Last Updated on STN: 20040224

AB Mechanisms that allow replicative DNA polymerases to attain high processivity are often specific to a given **polymerase** and cannot be generalized to others. Here we report a protein engineering-based approach to significantly improve the processivity of DNA polymerases by covalently linking the **polymerase** domain to a **sequence** non-specific dsDNA binding protein. Using Sso7d from Sulfolobus solfataricus as the DNA binding protein, we demonstrate that the processivity of both family A and family B polymerases can be significantly enhanced. By introducing point mutations in Sso7d, we show that the dsDNA binding property of Sso7d is essential for the enhancement. We present evidence supporting two novel conclusions. First, the fusion of a heterologous dsDNA binding protein to a **polymerase** can increase processivity without compromising catalytic activity and enzyme stability. Second, **polymerase** processivity is limiting for the efficiency of **PCR**, such that the fusion enzymes exhibit profound advantages over unmodified enzymes in **PCR** applications. This technology has the potential to broadly improve the performance of nucleic acid modifying enzymes.

L7 ANSWER 2 OF 43 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 2

ACCESSION NUMBER: 2004100956 EMBASE
TITLE: Multiplex **polymerase** chain reaction analysis of Glu-1 high-molecular-mass glutenin genes from wheat by capillary electrophoresis with laser-induced fluorescence detection.
AUTHOR: Salmanowicz B.P.; Moczulski M.
CORPORATE SOURCE: B.P. Salmanowicz, Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszynska 34, PL 60-479 Poznan, Poland. bsal@igr.poznan.pl
SOURCE: Journal of Chromatography A, (2 Apr 2004) 1032/1-2 (313-318).
Refs: 26
ISSN: 0021-9673 CODEN: JCRAEY
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The unique bread-making properties of wheat are closely correlated with composition and quantity of high-molecular-mass (HMW) glutenin subunits encoded by the Glu-1 genes. We report the development of a multiplex **polymerase** chain reaction (**PCR**) method to identify bread wheat genotypes carrying HMW glutenin allele composition of Glu-1 complex loci (Glu-A1, Glu-B1 and Glu-D1) by capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection. Two triplex primer sets of HMW glutenin subunit genes were examined. An automated and rapid CE-LIF technique is helpful in the multiplex **PCR** optimization **process.** Two fluorescent intercalating dyes (**EnhanCE**, and YO-PRO-1) are compared for detection of DNA fragments. Amplified DNA fragments of HMW glutenin Glu-1 genes were well separated both by agarose slab-gel electrophoresis and CE, and revealed minor differences between the **sequences** of 1Ax2*, 1Axnull, 1Bx6, 1Bx7, 1Bx17 and 1Dx5 genes. Moreover, CE technique requires samples of smaller volumes in comparison to slab-gel electrophoresis, and data can be obtained in less than 20min. There was a very high concordance in the assessment of the molecular size of **PCR**-generated DNA markers. Fast and accurate identification of molecular markers of Glu-1 genes by CE-LIF can be an efficient alternative to standard procedure separation for early selection of useful wheat genotypes with good bread-making quality.

L7 ANSWER 3 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-18677 BIOTECHDS
TITLE: Performing **polymerase** reactions e.g. **polymerase** reaction on target nucleic acid present in a solution having **polymerase** inhibitor, using **polymerase** that is fused to **sequence** -non-specific nucleic acid-binding domain; plasmid-mediated gene transfer and expression in host cell for recombinant Sso7d-DELTA Taq fusion protein production using **polymerase** chain reaction and DNA **sequencing**
AUTHOR: WANG Y; VANDER HORN P; XI L
PATENT ASSIGNEE: MJ BIOWORKS INC
PATENT INFO: WO 2003046149 5 Jun 2003
APPLICATION INFO: WO 2002-US38441 27 Nov 2002
PRIORITY INFO: US 2001-333966 28 Nov 2001; US 2001-333966 28 Nov 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-493408 [46]
AN 2003-18677 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Performing **polymerase** reactions e.g. **sequencing** reaction or quantitative **polymerase** reaction on target nucleic acid (T) that has DNA-binding fluorescent dye, using a **polymerase** (I) joined to **sequence**-non-specific nucleic acid-binding domain (D1) that binds to double-stranded nucleic acid, and **enhances processivity** of **polymerase** compared to identical **polymerase** not having (D1) fused to it.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) **Sequencing** a nucleic acid in an aqueous solution using an improved **polymerase**, involves contacting a target nucleic acid (T) with a **polymerase** (I), where the solution is of a composition that permits **sequence**-non-specific nucleic acid-binding domain (D1) to bind to (T) and the **polymerase** domain to extend a primer that is hybridized to (T); and incubating the solution under conditions in which the primer is extended by the **polymerase**; and (2) Performing a quantitative **polymerase** reaction on (T) present in a solution that comprises a DNA-binding fluorescent dye (a **polymerase** inhibitor) such that the yield from **polymerase** reaction is increased, involves contacting (T) with (I), where the solution is of a composition that permits (D1) to bind to (T) and the **polymerase** domain to extend a primer that is hybridized to (T); incubating the solution under conditions in which the primer is extended by the **polymerase**; and exposing the solution to a suitable excitation light and measuring fluorescence emission from the fluorescent dye.

BIOTECHNOLOGY - Preferred Method: The **polymerase** domain has thermally stable **polymerase** activity. The thermally stable **polymerase** domain is a DELTA Taq **polymerase** domain or comprises a Pyrococcus **polymerase** domain. (D1) specifically binds to polyclonal antibodies generated against Sso7d (a basic chromosomal protein from the hyperthermophilic archaebacteria Sulfologus solfatarius), and contains a 50 amino acid subsequence containing 50% amino acid similarity to Sso7d. Preferably, (D1) is Sso7d. The fluorescent dye is SYBR Green I.

USE - The method is useful for performing **polymerase** reactions e.g., **sequencing** a nucleic acid in an aqueous solution, and performing a quantitative **polymerase** reaction on (T) present in a solution that comprises a DNA-binding fluorescent dye (a **polymerase** inhibitor), such that the yield from the **polymerase** reaction is increased. The methods are useful for increasing the yield from a **polymerase** reaction on a target nucleic acid (a crude sample) present in a solution that comprises a **polymerase** inhibitor (claimed). The method is also useful for

performing **polymerase** reaction in the presence of contaminants e.g. a crude nucleic sample.

ADVANTAGE - The **polymerase** reactions are more efficient and yield more product than traditional polymerases. The binding domains dissociate double stranded nucleic acid at a very slow rate. Thus, they increase the processivity and/or efficiency of a modifying enzyme to which they are joined by stabilizing the enzyme-nucleic acid complex. The improved **polymerase** are not sensitive to the fluorescent dye i.e., not inhibited to the same extent as an unimproved **polymerase.** The improved polymerases are more tolerant to contaminants, and are thus advantageous over standard enzymes when performing **polymerase** reactions.

EXAMPLE - Construction of Sso7d-DELTA Taq fusion was carried out in which the **sequence**-non-specific double-stranded nucleic acid binding protein Sso7d was fused to the Thermus aquaticus polI DNA **polymerase** that is deleted at the N terminus by 289 amino acids (DELTA Taq). Sso7d is a basic chromosomal protein from the hyperthermophilic archaebacteria Sulfologus solfatarius. Based on the published amino acid **sequence** of Sso7d, seven oligonucleotides were used in constructing a synthetic gene encoding Sso7d. The oligonucleotides were annealed and ligated using T4 DNA ligase. The final ligated product was used as the template in a **PCR** reaction using two terminal oligonucleotides as primers to amplify the full-length gene. By design, the resulting **PCR** fragment contained a unique EcoRI site at the 5' terminus, and a unique BstXI site at the 3' terminus. In addition to encoding the Sso7d protein, the above **PCR** fragment also encoded a peptide linker with the amino acid **sequence** of Gly-Gly-Val-Thr positioned at the C terminus of the Sso7d protein. The synthetic gene of Sso7d was then used to generate a fusion protein in which Sso7d replaces the first 289 amino acid of Taq. The fragment encoding Sso7d was subcloned into a plasmid encoding Taq **polymerase** to generate the fusion protein, as follows. Briefly, the DNA fragment containing the synthetic Sso7d gene was digested with restriction endonucleases EcoRI and BstXI, and ligated into the corresponding sites of a plasmid encoding Taq. As the result, the region that encodes the first 289 amino acid of Taq was replaced by the synthetic gene of Sso7d. The plasmid (pYW1) allows the expression of a single polypeptide containing Sso7d fused to the N terminus of DELTA Taq via a synthetic linker composed of Gly-Gly-Val-Thr. The processivity of the fusion polymerases was then assessed. Processivity was measured by determining the number of nucleotides incorporated during a single binding event of the **polymerase** to a primed template. 40 nM of a 5' FAM-labeled primer (34 nt long) was annealed to 80 nM of circular or linearized ssM13mp 18 DNA to form the primed template. The primed template was mixed with the DNA **polymerase** of interest at a molar ratio of approximately 4000:1 (primed DNA:DNA **polymerase**) in the presence of standard **PCR** buffer (free of Mg++) and 200 micro M of each dNTPs. MgCl2 was added to final concentration of 2 mM to initiate DNA synthesis. At various times after initiation, samples were quenched with **sequencing** loading dye containing 99% formamide, and analyzed on a **sequencing** gel. The median product length, which was defined as the product length above or below which there were equal amounts of products, was determined based on integration of all detectable product peaks. At a **polymerase** concentration for which the median product length change with time or **polymerase** concentration, the length corresponds to the processivity of the enzyme. The Sso7d- DELTA Taq provided a median product length of 39-58 nucleotides, while DELTA Taq provided a median product length of 2-6 nucleotides. (49 pages)

L7 ANSWER 4 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-19183 BIOTECHDS
TITLE: Identifying polymorphisms using an error correcting assay that utilizes an improved generation of nucleic acid polymerases and multiplexing the assay;

DNA polymorphism detection using DNA microarray and DNA amplification

AUTHOR: WANG Y; FINNEY M; CHEN F
PATENT ASSIGNEE: MJ BIOWORKS INC
PATENT INFO: WO 2003046208 5 Jun 2003
APPLICATION INFO: WO 2002-US38435 27 Nov 2002
PRIORITY INFO: US 2001-334032 28 Nov 2001; US 2001-334032 28 Nov 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-505210 [47]
AN 2003-19183 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying (M1) a polymorphism using an error-correcting assay comprising contacting target nucleic acid with query **sequence** and probe oligonucleotide that specifically hybridizes to the nucleic acid, providing an error-correcting **polymerase**, incubating the assay where the probe is extended by the **polymerase**, where the labeled query nucleotide is cleaved from the probe when mismatched with the query **sequence**, and detecting the probe, is new.

DETAILED DESCRIPTION - The 3' nucleotide of the probe in the method cited above is a labeled query nucleotide and is attached to a discrete surface location. The method alternatively comprises, after the incubating step, providing a capture oligonucleotide attached to a discrete location and complementary to the extended product, hybridizing the extended product of the capture oligonucleotide or separating the extended products electrophoretically, and detecting the amount of label at the discrete location. The error-correcting **polymerase** comprises at least two heterologous domains, where a first domain that is a **sequence**-non-specific nucleic acid binding domain is joined to a second domain that is a **polymerase** domain, and where the **sequence** non-specific nucleic acid binding domain.

WIDER DISCLOSURE - Also disclosed are nucleic acids, peptides, probes, primers and solid support used in the method of the invention.

BIOTECHNOLOGY - Preferred Method: The **polymerase** is a family B **polymerase** that is a Pyrococcus **polymerase.** The error correcting **polymerase** is joined to a **sequence** non-specific nucleic-acid-binding domain that binds to double-stranded nucleic acid, and **enhances** the **processivity** of the **polymerase** compared to an identical **polymerase** not having the **sequence** non-specific nucleic-acid-binding domain fused to it, and is Pfu-Sso7d. The target nucleic acid is a **PCR** amplicon obtained using a **PCR** reaction performed with two unlabeled primers, where the query **sequence** is not present in either of the two primers, and is obtained using a **PCR** reaction performed with two unlabeled primers, where the query **sequence** is not present in either of the two primers, and further, where the **PCR** reaction is contacted with the probe oligonucleotide during amplification. The oligonucleotide probe is complementary to a region in the amplicon that does not comprise the two primers. The discrete surface location is on a microarray. The 3' query nucleotide is labeled with a quencher. The method further comprises a second probe oligonucleotide.

USE - M1 and the compositions are useful for detecting polymorphisms utilizing amplification and error correction.

EXAMPLE - The efficiency with which modified and unmodified error-correcting polymerases can amplify products from small numbers of input template copies were tested using real time **polymerase** chain reaction (**PCR**) in the presence of input template of the double-stranded DNA specific fluorescent dye SYBR Green 1 in a DNA engine opticon continuous fluorescence detection thermal cycling system. A 57 bp portion of the human cytochrome P450 gene CYP2D6 was amplified using primers F1 and R1 from a template containing a perfect match to both primers. The number of thermal cycles required for the fluorescence to reach a threshold value was recorded. A detectable amount of DNA is at least 2 standard deviations, and usually 5 or more standard deviations above the background noise level. In conditions employed in this example,

a detectable amount corresponds to approximately 1 ng of DNA. An efficient **polymerase** may be able to produce a detectable amount of DNA in a smaller number of cycles by more closely approaching the theoretical maximum amplification efficiency of **PCR.** (35 pages)

L7 ANSWER 5 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-15214 BIOTECHDS
TITLE: Novel cellulase protein with the biological activity of beta-glucosidase, for improving the characteristics of yeast dough or baked goods made from the dough, and to enhance aroma of fruit or finished wine products; recombinant cellulase production and purification useful for surfactant, food-additive and ethanol production
AUTHOR: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003027306 3 Apr 2003
APPLICATION INFO: WO 2002-US24242 30 Jul 2002
PRIORITY INFO: US 2001-957880 21 Sep 2001; US 2001-957880 21 Sep 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-371926 [35]
AN 2003-15214 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified cellulase protein, termed BGL3 polypeptide (I), with the biological activity of a beta-glucosidase, comprising a **sequence** (S1) fully defined in the specification, a **sequence** having at least 85%, 90% or 95% **sequence** identity to S1, or a substantially purified biologically active fragment of S1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated polynucleotide (II) encoding (I) derived from a fungal source, comprises a nucleotide **sequence** (S2) given in the specification or its complement, a **sequence** which encodes or is complementary to a **sequence** which encodes (I), or a nucleic acid **sequence** that hybridizes, under high stringency conditions to (S2), or its complement or fragment; (2) an expression construct (III) including a polynucleotide **sequence** having at least 85% **sequence** identity to S1 or being capable of hybridizing to a probe derived from (II) under conditions of intermediate to high stringency, or being complementary to a nucleotide **sequence** having at least 85% **sequence** identity to S1; (3) a vector (IV) including (III), or comprising (II), operably linked to control **sequences** recognized by a host cell transformed with the vector; (4) a host cell (V) transformed with (IV); (5) a recombinant host cell comprising (II), or a deletion or insertion or other alteration in the beta- glucosidase (bgl3) gene which inactivates the gene and prevents BGL3 polypeptide production; (6) producing an enzyme having beta-glucosidase activity; (7) a purified enzyme having beta-glucosidase activity prepared using (V); (8) an antisense oligonucleotide complementary to a messenger DNA that encodes a BGL3 polypeptide, where upon exposure to a beta-glucosidase-producing host cell, the oligonucleotide decreases or inhibits the production of beta- glucosidase by the host cell; and (9) a detergent composition comprising (I).

WIDER DISCLOSURE - Anti-BGL3 antibody, is also disclosed.

BIOTECHNOLOGY - Preparation: (I) is prepared by stably transforming a host cell with an expression vector comprising (II), cultivating the transformed host cell under conditions suitable for the host cell to produce the beta-glucosidase, and recovering the beta-glucosidase. The host cell is a filamentous fungi or yeast cell (claimed). Preferred Polynucleotide: The percentage identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. The hybridization is conducted at 42 degreesC in 50% formamide, 6 x SSC, 5 x Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS) and 100 micrograms/ml denatured carrier DNA

followed by washing two times in 2 x SSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42 degreesC. The polynucleotide is an RNA molecule, and the enzyme is derived from a Trichoderma source, preferably T. reesei. Preferred Host Cell: The host cell is a prokaryotic or eukaryotic cell.

USE - (I) is useful for improving the characteristics of a yeast dough or baked good made from such dough, by mixing at least about 10 ppm of a BGL3 with dough ingredients to form a dough mixture, and baking the dough mixture to form a baked good. (I) is useful for improving yeast bread dough or yeast roll dough or yeast bread or yeast roll, by mixing at least about 10 ppm of a BGL3 with bread or roll dough ingredients to form a dough mixture, shaping or panning the dough mixture, proofing the dough mixture, and baking the dough mixture to form bread or rolls. (V) is useful for expressing a heterologous polypeptide having beta-glucosidase activity in an Aspergillus sp., by providing a host Aspergillus with an expression vector comprising a polynucleotide encoding an Aspergillus beta-glucosidase signal **sequence** linked to a polynucleotide encoding a heterologous beta-glucosidase, thus encoding a chimeric polypeptide, cultivating the host Aspergillus under conditions suitable for the Aspergillus to produce the chimeric polypeptide, and recovering chimeric polypeptide thus produced (all claimed). The composition is useful as a household detergent, or laundry detergent, softening agent, and/or to improve the feel of cotton fabrics (e.g. stone washing or biopolishing), for degrading wood pulp into sugars e.g. for bio-ethanol production and/or in feed composition, and for the production of cellobiose and other cello-oligosaccharides. (I) is useful in food additives or wine **processing** to **enhance** the flavor or aroma, to enhance aroma of fruit or finished wine product, and for producing anti- BGL3 antibody. (II) is useful for generating bacterial chimeric surface proteins, allowing whole-cell immobilization onto cellulose filters or other fibrous solid supports, in the identification and characterization of related nucleic acid **sequences**, as hybridization probes for a cDNA library to isolate genes encoding naturally-occurring BGL3 from other fungal, bacterial or plant species, which have a desired level of **sequence** identity to bgl3 nucleotide **sequence**, and as probes to identify and **clone** out homologous nucleic acid **sequences** from related organism.

EXAMPLE - A cDNA fragment for use as a probe was isolated by extracting total RNA from mycelia of a Trichoderma reesei strain grown under conditions known to induce cellulose production and obtaining the polyadenylated (polyA) fraction from it. The polyA RNA was used to produce a cDNA pool which was then amplified using specific primers based on the bgl3 nucleic acid **sequence** given in the specification. Total RNA was isolated from the mycelia and Northern blots were performed to confirm cellulase expression and selected an optimal induction time for cellulase expression and corresponding RNA isolation. Messenger RNA (mRNA), having a poly (A) tail at the 3' end, was purified from total RNA. T. reesei RNA was used as template for RT-**PCR**. The mRNA was reverse transcribed to produce first strand cDNA. The cDNA subsequently served as template for **PCR** amplification of bgl3 cDNA **sequences** using specific oligonucleotide primers. Full length bgl3 cDNA nucleic acid **sequence** and its deduced amino acid **sequence** is fully defined in the specification.(49 pages)

L7 ANSWER 6 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-11178 BIOTECHDS
TITLE: New Beta-site APP cleaving enzyme (BACE) proteins and protein crystal, useful in designing compounds that inhibit or modulate BACE, in drug screening assays, and in identifying receptors;
involving baculo virus vector plasmid pFastBac1-mediated gene transfer and expression in host cell for use in drug screening
AUTHOR: YON J; CLEASBY A; BRUINZEEL W D; MASURE S L J; TICKLE I;

SHARFF A
PATENT ASSIGNEE: ASTEX TECHNOLOGY LTD; JANSSEN PHARM NV
PATENT INFO: WO 2003012089 13 Feb 2003
APPLICATION INFO: WO 2002-GB3461 26 Jul 2002
PRIORITY INFO: US 2001-308366 26 Jul 2001; US 2001-308366 26 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-239524 [23]
AN 2003-11178 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A new crystalline form of Beta-site APP cleaving enzyme (BACE) or its functional portion having an active site containing one or more ligands other than the natural substrate or the substrate that occurs naturally or physiologically within the active site.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a catalytic domain or a form of BACE for crystallization with the correct disulphide bonding that eliminates the need for refolding; (2) an apo-BACE crystal grown at or near the physiological pH of the enzyme or which can be soaked to give complexes; (3) a method for ligand screening or identification; (4) a computer-assisted method for identifying or designing potential ligands to fit within the catalytic domain of BACE or its functional portion; (5) a compound having a chemical structure selected using the above, where the compound is a BACE modulator; (6) a BACE protein or its functional portion comprising: (a) amino acid **sequences** of the catalytic domain that crystallize to the crystalline structure above, or to a structure that mimics the crystalline structure; or (b) one or more mutations or truncations to prevent glycosylation or facilitate crystallization and/or growth of ordered, well diffracting crystals, when compared to wild type BACE or BACE of Genbank accession number P56817; (7) an isolated nucleic acid molecule encoding above defined BACE protein or its functional portion; (8) a vector or cell comprising or expressing the nucleic acid molecule, and a nucleic acid molecule encoding an enhancer that enhances the particular vector or cell system the total amount of BACE produced and/or increases the fraction of processed protein; (9) a kit for producing the vector or cell containing separately packaged nucleic acid molecules comprising a BACE-protein encoding nucleic acid molecule and a nucleic acid molecule encoding the enhancer; (10) a method for obtaining a BACE protein; (11) a method for crystallizing a BACE protein or its functional portion; (12) a method for determining the crystal structure of a BACE protein or its functional portion by obtaining BACE protein crystals and obtaining their x-ray diffraction pattern; (13) a method for ligand screening and design or identification; (14) a ligand identified by the method of (4) or (13); (15) an assay comprising a BACE protein or its functional portion and means to determine whether a compound is a BACE modulator; (16) an antibody elicited by a BACE protein or its functional portion; (17) an inhibitor of a BACE protein or its functional portion; (18) compositions comprising the above inhibitor, ligand, or a product from the assay of (15); (19) a method for inhibiting BACE or the production of Abeta or its fragments, or treating AD in an individual by administering BACE protein inhibitor or its functional portion, or a ligand above; (20) a computer system for generating structures or performing rational compound or drug design for BACE or complexes of BACE with a potential modulator, where the system contains atomic coordinate data that defines the 3-dimensional structure of BACE or at least one of its sub-domain, or structure factor data for BACE derived from the atomic coordinate data; (21) a computer readable media with either atomic coordinate data that defines the 3-dimensional structure of BACE or at least one of its subdomain, or structure factor data for BACE derivable from the atomic co-ordinate data; and (22) a method of doing business by providing to a user the above defined computer system, computer readable media, 3-dimensional structure of BACE or at least one of its sub-domain, or a structure factor data for BACE derivable from the atomic coordinate.

BIOTECHNOLOGY - Preferred Crystal: The crystalline form of BACE or its functional portion are grown at a pH between about pH 5.6 and pH 5.8

The BACE crystal has a structure defined by the coordinates given in the specification. The BACE crystal has a space group of C2 and/or has cell dimensions of a= 236.63 Angstrom or 236.63 Angstrom +/- standard deviation (0.23 Angstrom) or 236.633 Angstrom +/- 3.03 Angstrom, b = 105.02 Angstrom or 105.02 Angstrom / standard deviation (0.2 Angstrom) or 105.02 Angstrom +/- 3.0 Angstrom, c = 62.59 Angstrom or 62.59 Angstrom +/- standard deviation (0.2 Angstrom) or 62.59 Angstrom +/- 3.03 Angstrom, and beta = 101.32 degrees or 101.32 degrees +/- standard deviation (0.2 degrees) or between 101 and 108 degrees with the asymmetric unit of the crystal containing three copies of BACE or cell dimensions a = 238.3 Angstrom or 238.3 Angstrom +/- standard deviation (0.2 Angstrom) or 238.3 Angstrom +/- 3.0 Angstrom, b = 107.4 Angstrom +/- standard deviation (0.2 Angstrom) or 107.4 Angstrom +/- 3.0 Angstrom, and c = 60.4 Angstrom or 60.4 Angstrom +/- standard deviation (0.2 Angstrom) or 60.4 Angstrom +/- 3.03 Angstrom, and beta = 101.89 degrees or 101.89 degrees +/- standard deviation (0.2 degrees) or between 101 degrees and 108 degrees, and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing, and/or having a space group transition from C2 to P21 together with an increase in the number of copies of the molecule in the asymmetric trait, while the cell dimensions and the packing of the P21 form are closely related to those of the C2 crystal form, on soaking the apo-BACE crystal with a ligand; and/or a BACE crystal having a resolution better than 3Angstrom; and/or a BACE crystal having the structure defined by the co-ordinates given in the specification. Preferred Method: In ligand screening or identification, the BACE protein or its functional portion is exposed to the test samples by co-crystallizing the BACE protein or its functional portion in the presence of one or more test samples. The BACE protein is soaked in a solution of one or more test samples. The computer assisted method for identifying or designing potential ligands to fit within the catalytic domain of BACE or its functional portion, comprises: (a) using a presumed computer consisting of a processor, a data storage system, an input device, and an output device by: (i) inputting into the programmed computer through the input device data comprising the 3-dimensional coordinates of a subset of the atoms in the BACE catalytic domain, optionally with structural information from ligand-BACE complexes to generate a data set; (ii) using the processor, comparing the data set to a computer database of chemical structures stored in the computer data storage system;(iii) using the computer methods, selecting from the database chemical structures having a portion that is structurally similar to the data set; (iv) using computer methods, constructing a model of a chemical structure having a portion that is structurally similar to the data set; and (v) outputting to the output device the selected chemical structures having a portion similar to the data set, and optionally synthesizing one or more of the selected chemical structures, and contacting the synthesized selected chemical structure with BACE to ascertain whether the synthesized chemical structure is a ligand that fits within the catalytic domain of BACE and/or inhibits BACE; or (b) providing the structure of BACE as defined by the coordinates in the specification, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the structure of the BACE given in the specification; or (c) providing the coordinates of at least 2 atoms of BACE (selected co-ordinates), providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the selected co-ordinates of BACE; or (d) providing the co-ordinates of at least a sub-domain of BACE, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the subdomain of BACE. The method optionally further comprises obtaining or synthesizing the chemical structure or candidate modulator, and contacting the chemical structure or candidate modulator with BACE to determine the ability of the chemical structure or candidate to interact with BACE, or obtaining or synthesizing the chemical structure or candidate modulator and forming a complex of BACE and the chemical structure or candidate modulator, and analyzing the complex to determine the ability of the chemical structure

or candidate modulator to interact with BACE. Obtaining a BACE protein comprises expressing a nucleic acid molecule encoding a BACE protein, or the nucleic acid molecule of the vector or cell above. Crystallizing a BACE protein or its functional portion comprises dissolving a BACE protein in a solvent and crystallizing the same either in the presence or absence of an inhibitor, where the method optionally includes producing the BACE recombinantly or by expression by a vector, recovering the BACE so produced, and growing crystals from the recovered BACE. The inhibitor is OM99-2. The method for ligand screening and design or identification comprises exposing the BACE crystals of a BACE protein or its functional portion to one or more test samples, and determining whether a ligand-BACE complex is formed, where the BACE functional portion has an unoccupied active site. The BACE is exposed to the test samples by either co-crystallizing the BACE or its functional portion in the presence of the one or more test samples or soaking the BACE or its functional portion in a solution of one or more test samples. Preferred BACE Protein: The BACE protein having one or more mutations or truncations when compared with Genbank accession P56817 has one or more mutation at amino acid 153, 172, 223 or 354, and one or more truncations, where each of the mutations is asparagine to glutamine and results in a BACE extending from Thr22 to Ser453, with reference to Genbank Accession P56817. Alternatively, all of the mutations are present, and there is a truncation resulting in a BACE extending from Thr22 to Ser453, with reference to Genbank Accession P56817. The protein further includes one or more of the following: a tag to facilitate purification; a non-BACE signal **sequence** to facilitate or increase secretion of the protein into cell culture medium; and a tag to allow differentiation of species arising from incomplete pro-peptide cleavage. The tag which facilitate purification is a HIS tag, the non-BACE signal **sequence** is a baculovirus signal **sequence**, and the tag that allow differentiation of species is a FLAG tag, where all of the tags that facilitate purification, the non-BACE signal **sequence** and the tag that allow differentiation are present. The BACE which comprises or consists of a **sequence** of 414 amino acids (P1) or an amino acid **sequence** having greater than 98.8% identity with P1. Preferred Nucleic Acid: The nucleic acid encoding a BACE protein or its functional portion has a reduced GC content via silent mutations from nucleotide **sequences** derived from wild type BACE that would also encode the BACE protein. The nucleic acid molecule encoding the BACE comprises a **sequence** of 1470 (S1), or a **sequence** having greater than 95.6% identity with S1. Preferred Vector or Cell: The vector or cell comprising the nucleic acid encoding a BACE protein is a viral or bacterial vector, a mammalian cell or a DNA plasmid. The vector is baculovirus vector, and the cell is an insect cell. The vector or cell further includes a nucleic acid molecule encoding an enhancer that enhances in the particular vector or cell system the total amount of BACE produced and/or increases the fraction of **processed** protein, where the **enhancer** is a prohormone convertase.

ACTIVITY - Neuroprotective; Nootropic.

MECHANISM OF ACTION - Beta-site APP cleaving enzyme inhibitor.

USE - Inhibitors of BACE protein or its functional portion is useful for preparing a composition or medicament for inhibiting BACE or the production of Abeta or its fragments, and in therapy for treating Alzheimer's disease (claimed). The BACE crystals and proteins may be used to design compounds that inhibit or modulate BACE, in drug screening assays, and in identifying receptors.

ADMINISTRATION - Compounds may be administered at a dose of about 100ng/kg-100 mg/kg per day, preferably 10 pg/kg-10 mg/kg per day, through oral, subcutaneous, parenteral, intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal or intranasal routes.

EXAMPLE - The synthetic Beta-site APP cleaving enzyme (BACE) catalytic domain **sequence** was constructed by a combination or oligonucleotide synthesis and overlap **polymerase** chain reaction (**PCR**). Mutations were inserted at specific sites within the BACE catalytic domain **sequence** during synthesis to reduce GC

content. The synthetic gene was then cut with SalI and NotI to generate a 1489 bp fragment which was then subcloned into the expression vector pFastBac1, and the DNA **sequence** was verified by standard DNA **sequencing** methods. The cDNA encoding human furin was cut by restriction enzyme to generate 3216 bp Sma1 Xma1 fragment that was then subcloned into the expression vector pFastBac Dual. Expressed BACE protein was purified by affinity chromatography on nickel agarose resin, and by anion exchange chromatography. Fractions corresponding to the BACE protein containing the peak were buffer exchanged on an XK-50 column. Protein was then eluted and fractions were pooled based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Peak fractions containing the required form of BACE protein were pooled based on SDS-PAGE. Purified BACE eluted from size exclusion column at the position expected for monomeric protein and was monodisperse when subjected to dynamic light scattering. Crystals of BACE were grown by the hanging drop vapor diffusion where 1 microlitres of protein solution and 1 microlitres of well solution were placed on a cover slip and equilibrated over 1 ml of well solution at 20 degrees Centigrade. protein concentration was 5 mg/ml in 20 mM HEPES, 150 mM NaCl, 1 mM DTT. Small prismatic crystals appeared after 2 days and grew to a maximum size of 0.2 mm x 0.1 mm x 0.1 mm after 2 weeks.(136 pages)

L7 ANSWER 7 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-27858 BIOTECHDS
TITLE: Novel isolated or recombinant polynucleotide encoding antigenic human interleukin-B50 **sequence** which is useful for treating T cell immunodeficiencies, chronic inflammation or tissue rejection, or cardiovascular conditions;
recombinant protein production and antagonist and agonist for use in disease therapy
AUTHOR: BAZAN J F; DE WAAL MALEFYT R; LIU Y; SOUMELIS V
PATENT ASSIGNEE: BAZAN J F; DE WAAL MALEFYT R; LIU Y; SOUMELIS V
PATENT INFO: US 2003099947 29 May 2003
APPLICATION INFO: US 2001-963347 25 Sep 2001
PRIORITY INFO: US 2001-963347 25 Sep 2001; US 1998-101318 21 Sep 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-777307 [73]
AN 2003-27858 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated or recombinant polynucleotide (I) encoding an antigenic polypeptide comprising at least 17 contiguous amino acids from the mature polypeptide having a fully defined human interleukin (IL)-B50 **sequence** (S1) of 149 or 159 amino acids as given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an expression vector (II) comprising (I); (2) a host cell (III) containing (II) including a eukaryotic cell; (3) forming a duplex with (I), involves contacting the polynucleotide with a probe that hybridizes, under stringent conditions, to at least 25 contiguous nucleotides of the coding portion having a fully defined **sequence** (S2) of 468 or 480 nucleotides as given in the specification; (4) a kit for detecting (I), comprising a polynucleotide that hybridizes, under stringent hybridization conditions, to at least 17 contiguous nucleotides of (I); (5) a binding compound (IV) comprising an antibody binding site which specifically binds to at least 17 contiguous amino acids from (S1); (6) a detection kit comprising (IV), and instructional material for the use of the binding compound for the detection, or a compartment providing segregation of the binding compound; (7) a substantially pure or isolated antigenic polypeptide (V) which binds to (IV), and further comprises at least 17 contiguous amino acids from (S1); and (8) modulating (M1) physiology or development of a cell or tissue culture cells involves contacting the cell with an agonist or antagonist or a primate IL-B50.

WIDER DISCLOSURE - The following are disclosed: (1) a fusion protein

comprising IL-B50 **sequence**; (2) composition comprising IL-B50 polypeptide; (3) kit comprising IL-B50 polypeptide; (4) proteins or peptides having substantial amino acid **sequence** identity with IL-B50 antigen; and (5) functional variants that block physiological response to IL-B50 polypeptide.

BIOTECHNOLOGY - Preferred Polynucleotide: (I) preferably encodes a mature polypeptide from (S1), (I) hybridizes at 55 degrees Centigrade, less than 500 mM salt, and 50% formamide to the coding portion having a **sequence** of (S2). Preferably (I), comprises at least 35 contiguous nucleotides of the coding portion of (S2). Preferred Kit: The probe in the kit is detectable labeled. Preferred Binding Compound: (IV) comprises an antibody binding site which is specifically immunoreactive with a polypeptide of **sequence** (S1), is raised against a purified or recombinantly produced human IL-B50 protein, or is a monoclonal antibody, Fab, or F(ab)2. The binding compound is an antibody molecule, is a polyclonal antiserum, is detectably labeled, is sterile, or is in a buffered composition. Preferred Polypeptide: (V) comprises at least 17 contiguous amino acid residues from a primate IL-B50 protein, is a soluble polypeptide, is detectably labeled, is in a sterile composition, is in a buffered composition, binds to a cell surface receptor, is recombinantly produced, or has a naturally occurring polypeptide **sequence.** (V) comprises at least 17 contiguous amino acids of (S1). Preferred Method: In (M1), the contacting is in combination with an agonist or antagonist of IL-7, or the contacting is with an antagonist, including binding composition comprising an antibody binding site which specifically binds an IL-B50.

ACTIVITY - Antiinflammatory; Immunosuppressive; Immunostimulant; Cardiant; Neuroprotective.

MECHANISM OF ACTION - CD11c+ dendritic cells activator; T cell proliferation activator; IL-B50 agonist or antagonist; IL-7 agonist or antagonist. Peripheral blood mononuclear cells (PBMC) were purified from buffy coats of healthy volunteers. The PBMC were incubated with anti-CD3, anti-CD14, anti-CD19, anti-CD56 monoclonal antibodies (mAbs), depleted from lineage+cells using magnetic beads and CD11c+ lineage-blood dendritic cell (DC) were subsequently isolated by cell sorting to reach a purity of more than 99%. Freshly sorted cells were cultured in RPMI 1640 containing 10% fetal calf serum (FCS) at 5 x 10 (to the power of 4)/100 microlitres in flat-bottom 96-well half-area culture plates or at 1 x 10 (to the power of 5)/200 microlitres in flat-bottom 96-well plates, with or without IL-B50. Freshly purified immature CD11c+ blood DC were known to spontaneously mature in culture. In the presence of IL-B50, this maturation **process** was dramatically **enhanced.** DC in culture formed tight and round clumps with fine dendrites visible at the periphery of each clump. The IL-B50-induced maturation was confirmed by analyzing the surface phenotype of DC using flow cytometry. Whereas IL-B50 slightly upregulated the expression of HLA-DR and CD86, it strongly induced the co-stimulatory molecules CD40 and CD80. This maturation process was accompanied by an increased viability of the DC. The T cell stimulatory capacity of CD11+ DC, cultured 24 hrs in medium alone or in the presence of IL-B50, was analyzed. DCs were co-cultured with 5 x 10 (to the power of 4) naive CD4+CD45RA+ allogeneic T cells at increasing DC/T cell ratios. As assessed by 3H-thymidine incorporation at day 5 of the co-culture, DC cultured with IL-B50 induced up to 10-fold stronger naive T cell proliferation as compared to DC cultured in medium. Additionally, the T cell stimulatory capacity of CD11+ DC, cultured with lipopolysaccharide (LPS), medium alone, IL-7, CD40-ligand and IL-B50, was compared by determining 3H-thymidine incorporation. IL-B50-activated DCs were more potent than DCs activated with CD40-ligand, IL-7 and LPS in inducing proliferation of allogeneic naive CD4 T cells.

USE - (I) is useful for making an antigenic polypeptide. (IV) is useful for binding an antigen in a biological sample, where it forms a binding compound:antigen complex. The biological sample is from a human, and where the binding compound is an antibody (claimed). (I) is useful for identifying genes, mRNA, cDNA which code for related or similar proteins, as well as DNAs which code for homologous proteins from

different species. (I) is also useful in forensic sciences to distinguish rodent from human, or as a marker for distinguishing between different cells exhibiting differential expression or modification patterns. (V) or (M1) is useful for treating abnormal medical conditions such as immune disorders e.g. T cell immunodeficiencies, chronic inflammation or tissue rejection, or cardiovascular or neurophysiological conditions. (IV) is useful in diagnostic applications, and as potent antagonists that bind to the antigen and inhibit functional binding, e.g. to a receptor which may elicit a biological response. (IV) is also useful in affinity chromatography for isolating (V), and for raising anti-idiotypic antibodies.

ADMINISTRATION - (IV), or the agonists or antagonists of IL-B50 are administered by intravenous, oral, intraperitoneal or intramuscular route in dosages ranging from 1 fM to 10 microMolar.

EXAMPLE - Interleukin-B50 (IL-B50) gene was derived from a cDNA library made from testes. A number of experiments were conducted in order to assess the signaling receptor complex for IL-B50, as well as the biological function of IL-B50. The cytokine human IL-B50 had as closest homologs human and mouse interleukin 7 (IL-7) and the recently described TSLP Sims, et al., (2000) J.Expt'l Med. 192:671-680). In mouse, both IL-7 and TSLP function as T- and B-cell growth and differentiation factors. The signaling receptor complexes for mouse IL-7 and mouse TSLP consist of two subunits, respectively mouse IL-7Ralpha and mouse Rgammac (common receptor for IL-2, IL-4, IL-7, IL-9, and IL-15) for mouse IL-7, and mouse IL-7Ralpha and mouse TSLPR (Rdelta1) for the mTSLP ligand Park, et al., (2000) J.Expt'l Med. 192:659-670). To identify the signaling receptor complex for human IL-B50, Ba/F3 cells were co-transfected with expression constructs for human IL-7Ralpha and an orphan human cytokine receptor known as Rdelta2, a subunit related to Rgammac and mTSLPR (Rdelta1). Co-transfected Ba/F3 cells showed a proliferative response in the presence of hIL-B50, but not with hIL-7 or medium. Ba/F3 cells transfected with either hIL-7Ralpha or hRdelta2 alone did not show a proliferative response with hIL-B50. Additionally, no cross-reactivity between mTSLP and the hTSLP receptor complex was observed. These findings established the signaling complex for human IL-B50 as consisting of hIL-7Ralpha and hRdelta2. The corresponding activation status of signal transducer and activator of transcription (Stat)5 and Stat3 was also measured in the various BaF3 cell populations. Both Stat5 and Stat3 were phosphorylated upon addition of hTSLP, but only when both hTSLPR and hIL-7Ralpha was present. In order to identify target cells capable of responding to IL-B50, a large panel of cDNA libraries was analyzed for the simultaneous expression of both hIL-7Ralpha and hRdelta2 using quantitative **polymerase** chain reaction (**PCR**). Results of the expression analysis showed that two receptor subunits were co-expressed primarily in activated dendritic cells, monocytes, and T cells indicating that these cell types respond to human IL-B50. IL-B50 was expressed in various tissue types, with high expression in the human lung. The spectrum of biological activities induced by IL-B50 was investigated based on the overlapping expression patterns of IL-B50 receptor components. cDNA was prepared from human monocytes cultured for 24 hrs in the presence of IL-B50 or IL-7, and the expression of 38 human chemokines and 20 human chemokine receptors were analyzed by quantitative real time **PCR.** IL-B50 (TSLP) and IL-7 influenced the expression of distinct sets of chemokines. IL-B50 enhanced the expression of TARC/CCL17, DC-CK1/TARC/CCL18, MDC/CCL22, and MIP3/CL19. IL-B50 did not stimulate human DCs to produce mRNA for IL-6, IL-12p40, TNF, monocyte chemoattractant proteins (MCP-1), MCP-4, Rantes and MIG, but did stimulate human DCs to produce mRNA for the chemokines TARC, MDC and MIP3. (54 pages)

L7 ANSWER 8 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:397022 CAPLUS
DOCUMENT NUMBER: 138:380394
TITLE: Method for enhancing the efficiency of an enzymatic purification process

INVENTOR(S): Sudor, Jan
PATENT ASSIGNEE(S): Genset S.A., Fr.
SOURCE: PCT Int. Appl., 10 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003042391	A1	20030522	WO 2002-IB4624	20021014

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-332580P P 20011113

AB This invention relates to methods for **enhancing** the efficiency of biol. **processes** that involve successive enzymic reactions. More specifically, the invention relates to methods for enhancing the efficiency of an enzymic purification process by increasing the temperature between successive enzymic purification steps.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:470345 CAPLUS
DOCUMENT NUMBER: 139:48113
TITLE: Reversibly modified thermostable enzymes for DNA synthesis and amplification in vitro
INVENTOR(S): Sobek, Harald; Greif, Michael
PATENT ASSIGNEE(S): Roche Diagnostics GmbH, Germany; F. Hoffmann-La Roche AG
SOURCE: Eur. Pat. Appl., 36 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1319719	A1	20030618	EP 2002-26712	20021130

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

JP 2003199592	A2	20030715	JP 2002-351736	20021203

PRIORITY APPLN. INFO.: EP 2001-128725 A 20011203

AB The invention relates to a composition comprising a first modified thermostable enzyme exhibiting 3'→5'-exonuclease activity but essentially no DNA **polymerase** activity and a second modified thermostable enzyme exhibiting DNA **polymerase** activity. The fidelity of an amplification **process** is **enhanced** by the use of this composition in an amplification process in comparison to the use of a single second enzyme in an amplification process. The first thermostable enzyme may be 3'→5'-exonuclease from Archaeoglobus fulgidus and the second thermostable enzyme may be DNA **polymerase** from Thermus aquaticus. The first and said second modified thermostable enzyme is

reversibly modified by an inhibiting agent which results in essentially complete inactivation of enzyme activity. The modifying agent may be a dicarboxylic anhydride such as citraconic anhydride or cis-aconitic anhydride. Incubation of said first and said second modified thermostable enzyme in an aqueous buffer at alkaline pH at a temperature less than 25° for 20 min results in no significant increase in the activity of the first and second modified thermostable enzyme, whereas incubation at a temperature greater than 50° in an aqueous buffer at alkaline pH results in ≥2-fold increase in enzyme activity in less than 20 min, thereby allowing formation of primer extension products.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 43 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2003372833 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12907710
TITLE: Insertion of the T3 DNA **polymerase** thioredoxin binding domain **enhances** the **processivity** and fidelity of Taq DNA **polymerase.**
AUTHOR: Davidson John F; Fox Richard; Harris Dawn D; Lyons-Abbott Sally; Loeb Lawrence A
CORPORATE SOURCE: Department of Pathology, University of Washington, Seattle, WA 98195, USA.
CONTRACT NUMBER: 5 T32 CA09437 (NCI)
CA78885 (NCI)
CA80993 (NCI)
SOURCE: Nucleic acids research, (2003 Aug 15) 31 (16) 4702-9.
Journal code: 0411011. ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20030809
Last Updated on STN: 20040130
Entered Medline: 20040129

AB Insertion of the T3 DNA **polymerase** thioredoxin binding domain (TBD) into the distantly related thermostable Taq DNA **polymerase** at an analogous position in the thumb domain, converts the Taq DNA **polymerase** from a low processive to a highly processive enzyme. Processivity is dependent on the presence of thioredoxin. The **enhancement** in **processivity** is 20-50-fold when compared with the wild-type Taq DNA **polymerase** or to the recombinant **polymerase** in the absence of thioredoxin. The recombinant Taq DNA pol/TBD is thermostable, **PCR** competent and able to copy repetitive deoxynucleotide **sequences** six to seven times more faithfully than Taq DNA **polymerase** and makes 2-3-fold fewer AT-->GC transition mutations.

=> d ibib abs l7 11-20

L7 ANSWER 11 OF 43 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2003498726 EMBASE
TITLE: Chronic Ethanol Ingestion Facilitates N-Methyl-D-aspartate Receptor Function and Expression in Rat Lateral/Basolateral Amygdala Neurons.
AUTHOR: Floyd D.W.; Jung K.-Y.; McCool B.A.
CORPORATE SOURCE: Dr. B.A. McCool, Dept. of Physiology and Pharmacology, Wake Forest Univ. School of Medicine, 115 S. Chestnut, Winston-Salem, NC 27157, United States. bmccool@wfubmc.edu
SOURCE: Journal of Pharmacology and Experimental Therapeutics,

(2003) 307/3 (1020-1029).
Refs: 40
ISSN: 0022-3565 CODEN: JPETAB
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
032 Psychiatry
040 Drug Dependence, Alcohol Abuse and Alcoholism
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Withdrawal anxiety after chronic alcohol is likely to contribute to drug seeking and relapse in alcoholics. The brain regions regulating fear/anxiety behaviors, especially neurotransmitter systems with acute ethanol sensitivity, are potential targets for chronic ethanol-induced adaptations. We have therefore examined N-methyl-D-aspartate (NMDA) receptors after chronic ethanol ingestion in rat lateral/basolateral amygdala. Whole cell patch-clamp measurements indicate that chronic ethanol ingestion significantly increased NMDA receptor current density. This enhanced NMDA receptor function was also associated with an increase in ifenprodil inhibition and a decrease in apparent calcium-dependent current inactivation. These findings suggest that NR2B-containing receptors may be specifically **enhanced** and suggest that **processes** dependent upon calcium influx through amygdala NMDA receptors may potentially be enhanced by chronic ethanol ingestion. We measured subunit mRNA expression to investigate possible molecular mechanisms that control functional receptor adaptations to chronic ethanol. Quantitative real-time reverse transcription-**polymerase** chain reaction (RT-**PCR**) demonstrated that NR1 subunit mRNA expression, but not NR2 or NR3 expression, was enhanced in samples from chronic ethanol-exposed animals. Single-cell RT-**PCR** was then used to confirm that NR2 mRNA expression was unaltered by chronic ethanol. Most GAD (-), presumed projection neurons expressed both NR2A and NR2B mRNAs, and this profile did not change during chronic ethanol exposure. Our results suggest that both transcriptional and nontranscriptional adaptations to chronic ethanol ultimately contribute to alterations in NMDA receptor function. Because amygdala NMDA receptors play a significant role in many learned fear behaviors, chronic ethanol-induced adaptations in these receptors may influence the expression of withdrawal anxiety.

L7 ANSWER 12 OF 43 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:119013 BIOSIS
DOCUMENT NUMBER: PREV200300119013
TITLE: **Cloned** transgenic cattle produce milk with higher levels of beta-casein and kappa-casein.
AUTHOR(S): Brophy, Brigid; Smolenski, Grant; Wheeler, Thomas; Wells, David; L'Huillier, Phil; Laible, Gotz [Reprint Author]
CORPORATE SOURCE: Ruakura Research Centre, AgResearch, Hamilton, New Zealand goetz.laible@agresearch.co.nz
SOURCE: Nature Biotechnology, (February 2003) Vol. 21, No. 2, pp. 157-162. print.
ISSN: 1087-0156 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Mar 2003
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AB To **enhance** milk composition and milk **processing** efficiency by increasing the casein concentration in milk, we have introduced additional copies of the genes encoding bovine beta- and kappa-casein (CSN2 and CSN3, respectively) into female bovine fibroblasts. Nuclear transfer with four independent donor cell lines resulted in the production of 11 transgenic calves. The analysis of hormonally induced milk showed substantial expression and secretion of the transgene-derived caseins into milk. Nine cows, representing two high-expressing lines, produced milk with an 8-20% increase in beta-casein, a twofold increase in kappa-casein levels, and a markedly altered kappa-casein to total casein

ratio. These results show that it is feasible to substantially alter a major component of milk in high producing dairy cows by a transgenic approach and thus to improve the functional properties of dairy milk.

L7 ANSWER 13 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-08660 BIOTECHDS
TITLE: Novel interleukin 1 beta converting enzyme-protease activating factor, Ipaf polypeptide useful for inducing or suppressing apoptosis, and identifying inhibitor or **enhancer** of Ipaf mediated caspase **processing***; vector-mediated recombinant protein gene transfer and expression in host cell for use in therapy**
AUTHOR: **ALNEMRI E S**
PATENT ASSIGNEE: **UNIV JEFFERSON THOMAS**
PATENT INFO: **WO 2002097117 5 Dec 2002**
APPLICATION INFO: **WO 2002-US21946 24 May 2002**
PRIORITY INFO: **US 2001-293597 25 May 2001; US 2001-293597 25 May 2001**
DOCUMENT TYPE: **Patent**
LANGUAGE: **English**
OTHER SOURCE: **WPI: 2003-129523 [12]**
AN **2003-08660 BIOTECHDS**
AB **DERWENT ABSTRACT:**

NOVELTY - An isolated Ipaf polypeptide (I) (or its functional fragment) having at least 90% amino acid identity with a ***sequence (S1) of 1024 amino acids fully defined in the specification, where the Ipaf polypeptide or its functional fragment induces or suppresses procaspase-1 activation or oligomerizes with procaspase-1 or Ipaf of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a polynucleotide **sequence** encoding (I) or its fragment; (2) an expression vector (III) comprising, a promoter, and (II) which is operably linked to the promoter; (3) a host cell (IV) containing (III); (4) an antibody (V) that specifically binds (I) or its fragment; (5) a cell expressing (V); (6) an inhibitor or enhancer (VI) identified using (I) and (IV); (7) manufacturing (VI), by using (I) and (IV), and derivatizing the inhibitor or enhancer; (8) manufacturing a compound, by providing (VI), derivatizing (VI), and optionally rescreening (VI) by using (I) and (IV); (9) producing (I); (10) an Ipaf polypeptide produced by the above method; (11) an antisense nucleic acid molecule (VII) comprising a nucleic acid **sequence** at least 12 nucleotides which is complementary to (II); (12) a ribozyme (VIII) capable of specifically cleaving (II); (13) a double-stranded RNA molecule (IX) comprising a nucleic acid **sequence** at least 21 nucleotides which is complementary to (II); (14) a gene delivery vehicle (X) comprising (II), and a regulatory **sequence**, operably linked to each other; (15) detecting Ipaf transcription level, by providing a polynucleotide probe comprising a **sequence** that specifically hybridizes to (II) or its complement, contacting a biological sample with the probe under hybridization conditions that permit duplex formation, detecting the presence of the duplex, and comparing detected levels with a control;(16) a kit for screening for agents that after apoptosis, comprising (I), (II), and instructions for use of the same; (17) a diagnostic kit comprising (V), and a polynucleotide probe comprising a portion of (II), where the polynucleotide probe specifically hybridizes to (II) or its complement, and instructions for detecting differences between the levels of probe-containing duplexes or antibody-bound polypeptide, in the test biological sample and a normal biological sample; and (18) a composition (XI) comprising (I), (II) and (V).

WIDER DISCLOSURE - Also disclosed are: (a) nucleic acid **sequence** which are same as (II); and (b) use of mutant and variant forms of (I) and (II).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions and for a time sufficient for expression (I) (claimed). Preferred Polypeptide: (I) is a human Ipaf, encoded by a **sequence** (S2) of 3075 bp defined in the specification or its degenerate variant.

(I) comprises a CARD domain, NBD domain and LRR domain. The CARD domain has at least 90% identity with amino acids 1-88 of S1, and mediates dimerization between (I) or its functional fragment derived from (S1) or with caspase-1/procaspase-1. The CARD domain comprises amino acids 1-88 of (S1) and encoded by nucleotides 1-264 of (S2) or its degenerate variant. The NBD domain has at least 90% identity with amino acids 163-457 of (S1) and capable of binding nucleotides. The NBD domain comprises amino acids 163-457 of (S1), encoded by nucleotides 487-1371 of (S2), or its degenerate variant. LRR domain has at least 90% identity with amino acids 656-1024 of (S1) and interacts specifically with another LRR domain, thus mediating protein-protein interactions. The LRR domain comprises amino acids 656-1024 of (S1), encoded by nucleotides 1966-3072 of (S2), or its degenerate variant. The functional fragment of (I) comprises amino acids 1-256, 1-328, 1-557, 1-601 or 1-661 of (S1), and induces apoptosis or constitutively induces activation of procaspase-1 or oligomerizes with procaspase-1 or (I). Functional fragment of (I) is encoded by nucleotides 1-768, 1-984, 1-1671, 1-1803 and 1-983, respectively of (S2), or its degenerate variant. (I) comprises at least 10 or 20 consecutive residues of (S1). Preferred Nucleic Acid: (II) comprises at least 20, 50 or 100 contiguous nucleotide residues of (S2). (II) is fused to a heterologous nucleotide **sequence** such as beta-galactosidase, beta-glucuronidase, green fluorescent protein (GFP), blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). (II) is fused in frame at either the N-terminal or C-terminal of the Ipaf with a nucleotide **sequence** encoding a tag such as histidine (His) tags, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (FLAG) tag, Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly (T7) tag, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Preferred Vector: In (III), the promoter is a constitutive or inducible promoter. Preferred Host Cell: (IV) is a bacterium, yeast cell, nematode cell, insect cell, plant cell or mammalian cell. Preferred Antibody: (V) has an affinity of at least 107 M, and specifically binds a CARD domain, NBD domain, or LRR domain. (V) is monoclonal, polyclonal, antibody fragment, single chain or humanized antibody. Preferred Vehicle: (X) is a virus, especially retrovirus or adenovirus. The nucleic acid is associated with a polycation. (X) further comprises a ligand that binds a cell surface receptor.

ACTIVITY - Antiinflammatory; Cerebroprotective; Antiparkinsonian; Neuroprotective; Nootropic; Vasotropic; Cardiant; Cytostatic; Anti-tumor; Immunosuppressive.

MECHANISM OF ACTION - Modulator of (I) (claimed); Induces apoptosis by activating caspase-1 pathway and modulation of inflammation through interleukin-1beta; Gene therapy. 293T cells were transfected with procaspase-1 plasmid plus beta-galactosidase plasmid and either empty vector or Ipaf constructs together with or without an active site mutant (C285A) procaspase-1 expression construct. 20 hours following transfection cells were fixed and the morphology of 5-bromo-4-chloro-3-indolylbeta-D-galactopyranoside-stained cells were examined by light microscopy. Ipaf-661, but not the full-length Ipaf, was able to dramatically enhance caspase-1-induced apoptosis in 293T cells. Expression of full-length Ipaf or Ipaf 1-661 had no effect on cell viability when expressed alone in 293T cells. These results indicated that Ipaf was a very potent activator of procaspase-1 and this resulted in massive apoptotic cell death.

USE - (I) and (VI) are useful for identifying an inhibitor or enhancer of an Ipaf mediated caspase processing. (I) is useful for identifying an inhibitor or enhancer of an Ipaf induced caspase-1 activity. (I) and (II) are useful for inducing or suppressing apoptosis in a cell. (IV) is useful for identifying an inhibitor or enhancer of an Ipaf mediated apopotosis. (VII), (VIII) and (IX) are useful for treating a mammal with a disease or disorder associated with (I) (all claimed). (I) or (II) is useful for regulating apopotosis, proliferation and inflammation, and for identifying apoptotic modulators as well as to diagnose and treat diseases and disorders involving cell proliferation,

apoptosis, inflammation or immune response. (I) is useful as a therapeutic or in various assays to screen for modulators of apoptosis, as an immunogen for raising antibodies which are useful for determining the presence or absence of various Ipaf isoforms in a sample. (II) and (III) are useful in gene therapy. (V) is useful for detecting and isolating (I), and in the identification of molecules that interacts with (I), for inhibiting or enhancing the biological activity of (I) and for diagnostic or therapeutic purposes. (VI) is useful for controlling over the cell death process or cytokine activation, and for treating diseases characterized by excessive or insufficient levels of apoptosis and/or inflammation, and neurodegenerative diseases including stroke, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and myocardial infarction, ischemia, and diseases of the liver. (XI) is useful for inhibiting or promoting apoptosis or procaspase-1 processing, and for treating cancer, tumor progression, hyperproliferative cell growth, immune disorder, autoimmune disease, neurodegenerative disease, ischemic injury, and hepatic injury.

ADMINISTRATION - (XI) is administered at a dose of 0.1-100 mg/kg, preferably 0.001-50 mg/kg, by oral, nasal, venous, intracranial, intraperitoneal, subcutaneous, or intramuscular routes.

EXAMPLE - Isolation of the interleukin 1beta converting enzyme-protease activating factor (Ipaf) cDNA was carried out as follows. To isolate genes having **sequence** similarity to the CARD domain of caspase-1, a nucleotide **sequence** encoding a CARD containing peptide (GenBank accession number AL121653) with homology to caspase-1 was found in the NCBI public genomic database using the TBLASTN program. Based on the predicted cDNA **sequence** of this gene, the complete cDNA of the new CARD-containing protein was isolated from a human peripheral blood leukocytes (PBL) cDNA library by a **polymerase** chain reaction (**PCR**) with two sets of primers corresponding to overlapping **sequences** of the coding region of the gene. The cDNA **sequence** of the gene was verified by nucleotide **sequencing.** The nucleic acid **sequence** of Ipaf was 3075 nucleotides fully defined in the specification, encoding a **sequence** of 1024 amino acids fully defined in the specification, and was termed as Ipaf with a molecular weight of 116.1 kDa. (120 pages)

L7 ANSWER 14 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-00754 BIOTECHDS
TITLE: Providing latency to a pharmaceutically active agent, involves associating a fusion protein comprising a latency associated peptide and a proteolytic cleavage site with the pharmaceutically active agent;
plasmid-mediated recombinant protein gene transfer and expression in mouse for disease gene therapy
AUTHOR: CHERNAJOVSKY Y; DREJA H S; ADAMS G
PATENT ASSIGNEE: QUEEN MARY and WESTFIELD COLLEGE
PATENT INFO: WO 2002055098 18 Jul 2002
APPLICATION INFO: WO 2002-GB68 9 Jan 2002
PRIORITY INFO: GB 2001-551 9 Jan 2001; GB 2001-551 9 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-575421 [61]
AN 2003-00754 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Providing (M1) latency to a pharmaceutically active agent involves associating a fusion protein comprising a latency associated peptide (LAP) and a proteolytic cleavage site with the pharmaceutically active agent, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid construct (I) comprising a first nucleic acid **sequence** encoding a pharmaceutically active agent, and a second nucleic acid **sequence** encoding a LAP, where a nucleic acid **sequence** encoding a proteolytic cleavage site is provided between the first and second nucleic acid **sequences**; (2) a cell

WEST Search History

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DATE: Thursday, April 08, 2004

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	DB=PGPB,USPT,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L1	(polymerase same (enhanc$ or increas$) same process$)	2393
☐	L2	L1 same (spermidine or single-stranded DNA binding protein or SSB or tau DNAX)	49
☐	L3	polymerase enhancer	31
☐	L4	55449603.pn. or 5534407.pn.	2
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	DB=PGPB,USPT,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L6	5449603.pn.	2

END OF SEARCH HISTORY

Perfect Match Polymerase Enhancer, Strategies In Molecular Biology (A Stratagene newsletter) 4, 38; Erlich, H. A., Gelfand, D. and Sninsky, J. J. (1991). Recent Advances in the Polymerase Chain Reaction, Science 252, 1643-1651; Lundberg, K. S. and Mathur, E. J. (1991). Optimization of Perfect Match Polymerase Enhancer for the Polymerase Chain Reaction, Strategies In Molecular Biology (A Stratagene newsletter) 4, 4-5].

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L3: Entry 12 of 31 File: PGPB Apr 12, 2001

DOCUMENT-IDENTIFIER: US 20010000266 A1
TITLE: Novel polypeptides and polynucleotides relating to the alpha- and beta-subunits of glutamate dehydrogenases and methods of use

Detail Description Paragraph:
88. The 5'-terminal NADP-GDH cDNA sequences were cloned using a modified anchored PCR procedure for the rapid amplification of cDNA ends (Frohman, M. A. [1990] In D. H. Gelford, J. J. Sninсky, T. J. White, eds, PCR Protocols, Academic Press, San Diego, Calif., pp 28-38; Jain, R., R. H. Gorner, J. J. Murtagh [1992] Biotechniques 12:58-59). A mixture of poly(A).sup.+RNA, used in the synthesis of the .lambda.ZAP library, was utilized to clone the 5' end of the NADP-GDH mRNA. One hundred nanograms of the mRNA mixture were combined with 10 ng of a gene-specific primer (5'-CTCAAAGGCAAGGAACTTCATG-3', SEQ ID NO. 8), designed to hybridize to the conserved region of NADP-GDH mRNAs, heated for 5 minutes, and chilled on ice. First strand DNA synthesis was performed using Superscript.TM. reverse transcriptase (BRL) according to the supplier's protocol. The terminated reverse transcription reaction was treated with one unit of ribonuclease H for 20 minutes at 37.degree. C., 5 minutes at 95.degree. C., and extracted once with chloroform:isoamyl alcohol (24: 1, v/v). Excess primers and dNTPs were removed by centrifugation at 2000 rpm through an Ultrafree-MC filterfuge tube (30,000 MW cutoff, Millipore) and the retentate was concentrated to 10 .mu.l on a Savant Speedvac. The first-strand synthesis products were combined with 10 .mu.L of tailing mix (1X tailing buffer [Promega Corp.], 0.4 mM dATP, 10 units terminal deoxytransferase) and incubated at 37.degree. C. for 10 minutes. The reaction mixture was heated to 95.degree. C. for 5 minutes, diluted to 0.5 mL with TE (pH 8), and utilized as a cDNA pool. A mixture of 5 .mu.L of the cDNA pool, 5 .mu.L of Vent.TM. polymerase 10X buffer (New England Biolabs), 200 .mu.M of each dNTP, 25 pmol of a gene specific primer (SEQ ID NO. 8), 5 pmol of the poly(dT) adaptor primer (5'-GGGTCGACATTCTAGACAGA- ATTCGTGGATCC (T).sub.18-3'; SEQ ID NO. 9), 0.2 units Perfectmatch.TM. DNA polymerase enhancer (Stratagene), and 1 unit of Vent.TM. polymerase (NEB) in 50 .mu.L was amplified according to Jain et al., supra. The PCR products were purified away from the excess primers by centrifugation at 2,000 rpm through an Ultrafree-MC unit. The retentate was collected and subjected to two more rounds of amplification using a new nested gene specific primer at each step (5'-GGACGAGTACTGCACGC-3', SEQ ID NO. 10; 5'-GATCTCGGTCAGCAGCTG-3', SEQ ID NO.11, respectively) and an adaptor primer (5'-GGGTCGACATTCTAGACAGAA-3'; SEQ ID NO. 12). PCR amplifications were performed in a Model 480 thermocycler (Perkin-Elmer Cetus), and all custom oligonucleotides were synthesized by the ICBR DNA synthesis facility, University of Florida. The standard PCR reaction mixture consisted of 10 .mu.L of 10X Vent.TM. polymerase buffer, 100 .mu.M of each dNTP, 0.4 units of Perfectmatch.TM., 50 pmol of each primer, 1 unit Vent.TM. DNA polymerase in a 100 .mu.l reaction volume. The 5' RACE-PCR products were gel purified, subcloned into the SmaI site of pUC 18, and transformed into E. Coli DH5.alpha. for further characterization. RACE PCR identified two 5' cDNA clones, which overlapped with the previously identified pBGDc 53 clone, that differed by a 42 nt insert identified in one clone designated pRGDc 60 (SEQ ID NO. 13) and lacking in the second cDNA designated pRGDc 61 (SEQ ID NO. 14).

Other Reference Publication (79):
Lundberg, K. S. and Mathur, E. J. (1991). Optimization of Perfect Match Polymerase Enhancer for the Polymerase Chain Reaction, Strategies In Molecular Biology (A Stratagene newsletter) 4, 4-5, Stratagene, La Jolla, CA.

Other Reference Publication (82):
Nielson, K., Wilbanks, A., Hansen., C. and Mathur, E. J. (1991). Improve Specificity of Long Amplification Products with Perfect Match Polymerase Enhancer, Strategies In Molecular Biology (A Stratagene newsletter) 4, 38, Stratagene, La Jolla, CA.

First Hit Fwd Refs

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L2: Entry 15 of 49 File: USPT Sep 9, 2003

DOCUMENT-IDENTIFIER: US 6617114 B1
TITLE: Identification of drug complementary combinatorial libraries

Detailed Description Text (231):
Ertl and Powell (1992) showed that the HCMV polymerase and processivity factor, purified as recombinant proteins from baculovirus-infected Sf9 cells, cooperate in a primer extension reaction, and they further demonstrated that the processivity factor stimulated the activity of the polymerase in the assay. Similarly, Weiland et al., (1994) have shown that recombinant HCMV UL44 processivity factor produced in E. coli can enhance the activity of HCMV DNA polymerase in a primer extension assay. We will implement the origin-independent primer extension assay in the HCMV system as follows. The coding sequence for the HCMV polymerase (UL54) and single-stranded DNA-binding protein (UL57) will be amplified from HCMV genomic DNA as described above for the processivity factor (UL44). The UL54 and UL57 coding regions will be validated by automated DNA sequence analysis, and cloned into pBlueBacHis2 (Invitrogen). The plasmids will then be used to construct baculovirus recombinants for expression of the replication proteins in Sf9 insect cells. Expressed proteins carry two tags at their 5' ends, one the Xpress leader peptide (Asp Leu Tyr Asp Asp Asp Asp Lys) (SEQ ID NO:178) is easily detected with a monoclonal antibody in ELISA assays and the other includes a six histidine binding site that has a high affinity for divalent cations. Nickel-chelating resins will allow us to purify the recombinant proteins in one step. The HCMV equivalent of the HSV-1 replication assay will then be optimized using purified proteins.

(II) comprising (I); (3) a protein (III) which is the expression product of (I); (4) a fusion protein (IV) comprising a LAP and a proteolytic cleavage site, where (V) is associated with a pharmaceutically active agent; (5) a pharmaceutical composition (V) comprising (I) or (IV) and a pharmaceutically acceptable carrier; (6) preparation of (IV); and (7) a kit of parts (VI) comprising (I) or (IV) and an administration vehicle.

WIDER DISCLOSURE - Also disclosed as new are the following: (1) a nucleic acid encoding (IV); and (2) a composition comprising (III).

BIOTECHNOLOGY - Preparation: (IV) is prepared recombinantly by expression in a host cell, purification of the expressed fusion protein and association of a pharmaceutically active agent to the purified fusion protein by chemical cross linking (claimed). Preferred Construct: In (I), LAP comprises the precursor peptide of transforming growth factor (TGF)beta-1,2,3,4 or 5. The proteolytic cleavage site is a matrix metalloproteinase (MMP) cleavage site. The pharmaceutically active agent is a growth factor, differentiation factor, cytokine, chemokine, trophic factor, cytokine inhibitor, cytokine receptor, free-radical scavenging enzyme, pro-drug converting enzyme, peptide mimetic, protease inhibitor, tissue inhibitor of metalloproteinase sub class, inhibitor of serine protease, chemotherapeutic agent or peptide nucleic acid **sequence**. The first nucleic acid **sequence** encodes the protein interferon (IFN)beta. (I) is in the form of a vector.

ACTIVITY - Antiinflammatory; Osteopathic; Antiarthritic; Dermatological; Antirheumatic; Neuroprotective; Antiatherosclerotic; Cytostatic. DBA/1 mice were immunized with collagen type II (CII) and 3 weeks later were boosted with CII in incomplete Freund's adjuvant. 100 microg plasmid DNA in phosphate buffered saline (PBS) was injected intramuscularly at 3 sites in the quadriceps, on the day of arthritis onset and mice were scored every other day for clinical arthritis and hid paw swelling was measured with calipers. In a collagen induced arthritis model (CIA), the relative effectiveness of the latent cytokine (LAP-mIFNbeta) versus the active versions (PorcLAP-mIFNbeta and mIFNbeta-LAP) was measured. The latent LAP-mIFNbeta showed greater efficacy than either of the active moieties, mIFNbeta-LAP or PorcLAP-IFNbeta, as compared with the control treated with pCDNA3 empty plasmid vector. It was found that when delivered by gene therapy by intramuscular injection the latent cytokine was more efficacious in the treatment of the established disease.

MECHANISM OF ACTION - Gene therapy (claimed).

USE - M1 is useful for providing latency to a pharmaceutically active agent. (I) is useful for treatment of a patient such as a human or non-human animal by administering to the patient a therapeutically effective amount of (I), where the treatment is the treatment of an inflammatory disorder or gene therapy. (I) is useful in medicine or in the manufacture of a medicament for the treatment of an inflammatory disorder. (IV) is useful for treating a patient, for use in medicine or in the manufacture of a medicament for the treatment of an inflammatory disorder. (IV) is useful for providing latency to a pharmaceutically active agent or for providing site-specific activation of a latent biologically active agent (claimed). M1 is useful for overcoming toxic effects of systemic administration of potent biological agents. (I) or (III) is useful in veterinary medicine for treatment/prophylaxis of domestic animals including horses and companion animals (e.g., cats and dogs) and farm animals. (I) or (IV) is useful for treating inflammatory disorder such as osteoarthritis, scleroderma, renal disease, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, atherosclerosis, or cancer.

ADMINISTRATION - (V) is administered at a dose of 0.01 mg/kg body weight, preferably 1 mg/kg by oral, topical, intravenous, intramuscular, intranasal or intradermal route. (I) or (IV) is administered by intravenous, intradermal, intramuscular or oral route.

EXAMPLE - Construction of human latency associated peptide (LAP)-murine interferon-beta (mIFN-beta) fusion protein was as follows. GS-matrix metalloproteinase (MMP)-GS linker was **cloned** into EcoRI-NotI sites of pcDNA3. A vector was constructed by inserting the

GS-MMP-GS linker into EcoRI-NotI cleaved pcDNA3. pcDNA3 was an expression vector which comprised the human cytomegalovirus immediate early promoter and **enhancer**, together with RNA **processing** signals allowing transcription. Double stranded deoxyoligonucleotide coding for the **sequence** GGGGSPLGLWAGGGS was designed using Sense oligo: 5'-AATTCGGGGGAGGCGGATCCCCGCTCGGGCTTTGGGCGGGAGGGGGCTCAGC-3', and antisense oligo: 5'-GGCCGCTGAGCCCCCTCCCGCCCAAAGCCCGAGCGGGGATCCGCCTCCCCCG-3'. Synthetic deoxyoligonucleotides were obtained from Life Technologies Limited. Annealed deoxyoligonucleotides were **cloned** into EcoRI-NotI cleaved pcDNA3. The recombinant **clone** lost its EcoRV site and gained an additional BamH1 site. Plasmid **clones** were assessed by Southern blot hybridization with end labeled oligos. The **clone** was referred to as GS-MMP-GS. Restriction enzymes and DNA modifying enzymes were also obtained. Construction of LAP transforming growth factor beta (TGFbeta) at NH2 end followed by GS-MMP-GS and mature IFNbeta was as follows. A vector comprising LAP (TGFbeta) followed by GS-MMP-GS and mature IFNbeta was constructed as follows. LAP from TGFbeta as a 5' unit (with signal peptide) with HindIII and EcoRI ends was **cloned** by **polymerase** chain reaction (**PCR**) from plasmid TGFbeta-Babe neo using the primers 5'-CCAAGCTTATGCCGCCCTCCGGGCTGCGG-3' and CCGAATTCGCTTTGCAGATGCTGGGCCCT-3'. After **PCR**, the product was phenol extracted, ends filled-in with Klenow and digested with HindIII and EcoRI. The 820 base pair product was **cloned** into GS-MMP-GS plasmid cut with the same enzymes. The **clone** was referred to as TGFbeta-GS-MMP-GS linker. Mature mIFNbeta (from mouse) with 5' NotI and 3' XbaI sites was synthesized by **PCR** from **clone** Aphrodite using the primers: 5'-CGCGGCCGCAATCAACTATAAGCAGCTCCAG-3', and 5'-GGTCTAGATCAGTTTTGGAAGTTTCTGGTAAG-3'. After **PCR**, the fragment was phenol extracted, ends filled-in with Klenow and digested with NotI and XbaI. The LAP-mIFNbeta **clone** was obtained by **cloning** the fragment into the Not1 and XbaI sites of TGFbeta-GS-MMP-GS linker plasmid. (65 pages)

L7 ANSWER 15 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-17871 BIOTECHDS
TITLE: New variants of fibroblast growth factor, useful for treating skeletal disorders including osteoporosis, malignancies and to enhance wound and fracture healing; human, bird or mouse recombinant protein for use in diagnosis, therapy and drug screening
AUTHOR: BOGIN O; ADAR R; YAYON A
PATENT ASSIGNEE: PROCHON BIOTECH LTD
PATENT INFO: WO 2002036732 10 May 2002
APPLICATION INFO: WO 2000-IL962 31 Oct 2000
PRIORITY INFO: IL 2000-139380 31 Oct 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-479754 [51]
AN 2002-17871 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An active variant (I) of a fibroblast growth factor (FGF) having at least one mutation in the beta-8-beta-9 loop, having enhanced specificity for one receptor subtype compared to the corresponding wild type FGF, by decreasing the biological activity mediated by at least one receptor subtype while retaining the activity mediated through another receptor subtype, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a polynucleotide **sequence** (II) encoding (I); (2) a vector (III) comprising (II); (3) a host cell transfected with (III); and (4) a pharmaceutical composition (IV) comprising (I).

BIOTECHNOLOGY - Preferred Variant: FGF is FGF-1 to FGF-23 or FGF-CX. (I) is an FGF-9 variant from human, mouse or chicken. (I) has less than 30, preferably less than 22 amino acid residues at the N-terminus or less than 15 residues at the C-terminus extending beyond the core structure.

(I) totally has less than 175 amino acid residues. (I) includes heterologous **sequences** at the N-terminus or C-terminus.
Preferred Composition: (IV) comprises (I) further conjugated to a cytotoxic drug.

ACTIVITY - Cytostatic; Vulnerary; Osteopathic; Antiarthritic. (I) was tested for its effect on bone healing. The tested compounds consisted of FGF-9 or its variant (L37M-W144G-FGF9) in a polymeric scaffold which facilitated bone growth. The treatment group consisted of osteotomy without treatment, osteotomy treated with 0.2 ml of hyaluronic acid (HA) alone, osteotomy treated with 0.2 ml HA containing 20 microgram FGF-9, or osteotomy treated with 0.2 ml HA containing 20 microgram FGF-9 variant. A 0.6 cm radial gap osteotomy was created under anesthesia with rotary saw in both ulnas of each animal. About 1 ml of HA or HA containing the tested compounds was administered by injection into the gap. The periosteum, which was not resected during the surgery, was used to close the gap. Fracture healing was radiologically evaluated every week up to 4 weeks (p.o.). An X-ray closure of both limbs in a lateral position was taken. The autoradiograph analysis of ulnas in the various treatment groups, performed 4 weeks post surgery showed that the bone union was a time dependent process and the course of fracture healing was dependent upon the given treatment. Callus formation was detected as early as one week post osteotomy in fracture treated with the FGF-9 variant. This **process** was **enhanced** during the second week. In control animals callus formation was barely detected in at the same time intervals. Histological specimens of bone treated with the various samples were prepared. Quantitative bone mineral content measurements by DEXA were performed at the defect site for the various treatments at 4 weeks post osteotomy. The data indicated that a single local injection of 20 microgram L37M-W144G-FGF9 in combination with HA as a scaffold promoted healing of the bone defect by stimulating callus formation.

MECHANISM OF ACTION - Regulator of vasculogenesis or angiogenesis.

USE - (I) is useful for preparing a medicament and for treating a disease or disorder related to normal or abnormal FGF receptors (FGFRs), especially skeletal disorders, cancer, to enhance bone fracture healing or bone growth processes and wound healing processes (claimed). (I) is useful in detection and treatment of various FGFR related disorders including skeletal disorders e.g. achondroplasia, hypochondroplasia, and osteoporosis, and cartilage defects, multiple myeloma, epithelial cancers such as transitional cell carcinoma of the bladder and cervical carcinoma. The novel mutants are useful in high expression systems suitable for pharmaceutical production, targeting of drugs or other agents to tissues and cells having specific FGFR subtypes, and serve as template for the formation of improved agonists and antagonists of FGFRs in various disorders such as skeletal disorders and cancer.

ADMINISTRATION - Administered by parenteral administration through intra-articular, intravenous, intramuscular, subcutaneous, intradermal, or intrathecal routes. (I) is formulated for administration to the site of a bone fracture (claimed). Dosage not specified.

ADVANTAGE - (I) has enhanced receptor specificity, more stable, and higher in vivo activity.

EXAMPLE - Construction of p89BS was performed by a series of digestions of T. brockii (TB) genomic DNA, with restriction endonucleases. The TB adh gene was located on an EcoRI digest (2700 base pairs (bp)), and the altered fragment was first **cloned** into the EcoRI site of pBluescriptII. XbaI digestion of a positive **clone** produced a smaller (1673 bp) DNA fragment containing the entire TB adh gene, which was ligated to XbaI-digested pBluescriptII to form the plasmid pBS-M105/2. PBS-P89 was a deletion mutant in which the upstream region was limited to 89 bases preceding the initiation codon for the TB adh gene. This shortened fragment was **cloned** into the SacI-XbaI sites of pBluescriptII SK(+). Construction of the fibroblast growth factor-9 (FGF-9) variants was performed using **polymerase** chain reaction (**PCR**) technique. Three constitutive **PCR** reactions were performed, where the variation or variations were introduced into the gene by amplifying DNA fragments from both ends of

the mutation site(s). To produce L37M-W144G-FGF9 DNA, WG-for (5'-CGAAGAAAACGGGTATAATACGTAC-3') and FGF9(Stop-Back) (5'-AGCTGGATCCTCAACTTTGGCTTAGAATATCC-3') primers were used for the first **PCR** reaction. For the second **PCR** reaction WG-back (5'-GTACGTATTATACCCGTTTTCTTCG-3') and L37M-for (5'-ACGTGACCATATGGGTCAGTCCGAAGCAG-3') were used. The amplified DNA fragments were combined and served as the template for an additional **PCR** reaction. The purified **PCR** fragment was digested with NdeI and BamHI, and ligated into the p89BS construct. (74 pages)

L7 ANSWER 16 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-09254 BIOTECHDS
TITLE: Novel regulatory elements including nucleic acid encoding hybrid alpha-lactalbumin promoter or mutant RNA export element, for expressing one or more proteins e.g. antibodies, pharmaceutical proteins in host cells;
promoter, enhancer, virus vector expression in host cell useful for G-protein coupled receptor expression and in gene therapy
AUTHOR: BLECK G T
PATENT ASSIGNEE: GALA DESIGN INC
PATENT INFO: WO 2002002783 10 Jan 2002
APPLICATION INFO: WO 2000-US20714 3 Jul 2000
PRIORITY INFO: US 2000-215851 3 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-154749 [20]
AN 2002-09254 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A novel regulatory element (I) chosen from a nucleic acid encoding a hybrid bovine/human alpha-lactalbumin (alpha-LA) promoter/enhancer, having a 2101 base pair **sequence** (S1), fully defined in the specification, encoding a mutant RNA export element (pre-mRNA **processing enhancer**, PPE), comprising a 244 base pair **sequence** (S2), fully defined in the specification, or their hybridizable **sequences**, is new.

DETAILED DESCRIPTION - A novel regulatory element (I) chosen from a nucleic acid encoding a hybrid bovine/human alpha-lactalbumin (alpha-LA) promoter/enhancer, having a 2101 base pair **sequence** (S1), fully defined in the specification, encoding a mutant RNA export element (pre-mRNA **processing enhancer**, PPE), comprising a 244 base pair **sequence** (S2), fully defined in the specification, or their hybridizable **sequences**, is new. (I) comprises: (a) a nucleic acid **sequence** encoding hybrid bovine/human alpha-LA promoter or its hybridizable **sequence**, which contains **sequences** derived from two mammalian sources and causes mammary specific gene expression; (b) a nucleic acid **sequence** encoding mutant RNA export element or its hybridizable **sequence** which comprises ATG **sequences** that have been mutated at one of the positions corresponding to nucleic acid residues 4, 112, 131 and 238 of (S2); or (c) a nucleic acid **sequence** encoding a internal ribosome entry site (IRES) coding **sequence** and a signal peptide coding **sequence**, which are adjacent to one another. INDEPENDENT CLAIMS are also included for the following: (1) a vector (II) comprising (I); (2) a host cell (III) comprising (II); and (3) an antibody produced by introducing a vector comprising exogenous genes encoding immunoglobulin genes into a host cell.

BIOTECHNOLOGY - Preferred Nucleic Acid: The PPE element is a WPRE (Woodchuck hepatitis virus postregulatory element). The signal peptide is alpha-casein, human growth hormone or alpha-LA signal peptide. Preferred Vector: (II) is a retroviral vector.

USE - (I) and (II) are useful for expression of proteins of interest in a host cell. A vector comprising at least two exogenous genes encoding a protein operably linked to a bovine/human hybrid alpha-LA promoter, where the genes are arranged in a polycistronic **sequence**

separated by an IRES/bovine alpha-LA promoter signal peptide, and a mutant PPE element, is introduced into a host under conditions so that expression of the protein encoded by the exogenous gene is expressed. The regulatory **sequences** and vector are useful for producing an immunoglobulin (Ig), preferably secretory Ig, where a vector comprising a first and second exogenous genes which encode first and second Ig chain, separated by an IRES is introduced into a host cell under conditions so that the immunoglobulin chains are expressed. One of the first and second Ig chains is an Ig light chain such as kappa or lambda light chain, and the other is an Ig heavy chain such as alpha, mu, gamma, delta or epsilon heavy chain. The vector further comprises a bovine alpha-LA signal peptide and bovine/human hybrid alpha-LA promoter. The first and second antibody chains are expressed at a ratio of 0.9:1.1. The vector is a pseudotyped retroviral vector or a plasmid vector. (All claimed). (I) and (II) are useful in the expression of one or more proteins such as erythropoietin, alpha-interferon, alpha-1 proteinase inhibitor, angiogenin, antithrombin III, beta-acid decarboxylase, human growth hormone, bovine growth hormone, porcine growth hormone, human serum albumin, beta-interferon, calf intestine alkaline phosphatase, cystic fibrosis transmembrane regulator, factor VIII, factor IX, factor X, insulin, lactoferrin, tissue plasminogen activator, myelin basic protein, insulin, proinsulin, prolactin, hepatitis B antigen, immunoglobulins, monoclonal antibody CTLA4 Ig, Tag72 monoclonal antibody, Tag 72 single chain antigen binding protein, protein C, cytokines and their receptors, hormones, von Willebrands factor, lung surfactant, serum albumins, DNase, vascular endothelial growth factor, receptors for hormones or growth factors, rheumatoid factors, nerve growth factors, transforming growth factor, insulin-like growth factor, CD proteins, osteoinductive factors, immunotoxins, bone morphogenetic protein, interferons, colony stimulating factors, interleukins, superoxide dismutase, T-cell receptors, surface membrane proteins, viral antigens, transport proteins, addressins, regulatory proteins, antibodies, chimeric proteins, and their fragments. The vectors are particularly useful for expressing G protein coupled receptors and other transmembrane proteins. The retroviral vectors are useful for expressing proteins in mammalian tissue culture host cells, including rat fibroblast cells, bovine kidney cell and human kidney cells.

EXAMPLE - The alpha-lactalbumin (alpha-LA) MN14 construct contained 5'-MoMuLV (Moloney murine leukemia virus) long terminal repeat (LTR), MoMuLV extended viral packaging signal, neomycin phosphotransferase gene, bovine/human alpha-LA hybrid promoter, double mutated pre-mRNA **processing enhancer** (PPE) element, MN14 heavy chain signal peptide, MN14 antibody heavy chain, internal ribosome entry site (IRES) from encephalomyocarditis virus/bovine alpha-LA signal peptide, MN14 antibody light chain, WPRE (Woodchuck hepatitis virus postregulatory element) **sequence** and 3' MoMuLV LTR. The expression of MN14 antibody was controlled by the hybrid alpha-LA promoter. The MN14 heavy chain gene and light chain gene were attached together by IRES **sequence**/bovine alpha-LA signal peptide. The mutated PPE **sequence** was contained between the RNA CAP site and the start of the MN14 protein coding region. ATG **sequences** within the PPE element were mutated to prevent unwanted translation initiation. For construction of the bovine/human alpha-LA promoter, human genomic DNA was isolated and purified. A portion of human alpha-LA promoter was **polymerase** chain reaction (**PCR**) amplified and double digested with NdeI and EcoRI. Plasmid pKBaP-1 containing the bovine alpha-LA 5' flanking region attached to a multiple **cloning** site was also double digested with NdeI and EcoRI. The human fragment was ligated to the bovine fragment of the promoter that was removed from the pKBaP-1 plasmid during the double digestion. The resulting plasmid was confirmed by DNA **sequencing** to be a hybrid of the bovine and human alpha-LA promoter/regulatory regions. Attachment of the MN14 light chain gene to the IRES alpha-LA signal peptide was accomplished by **PCR** amplifying MN14 light chain from the vector pCRMN14LC. The resulting **PCR** product was digested with HincII and BglII and

cloned directly into the IRES-signal peptide plasmid that was digested with NaeI and BglII. The vector pCRMN14HC was digested with XhoI and NruI to remove a 500 base pair fragment. **PCR** was then used to amplify the same portion of the MN14 heavy chain construct that was removed by the XhoI-NruI digestion. This amplification also mutated the 5' end of the gene to add a better Kozak **sequence** to the **clone.** The Kozak **sequence** was modified to resemble the typical immunoglobulin (Ig)G Kozak **sequence.** The **PCR** product was digested with XhoI and NruI and inserted back into the previously digested plasmid backbone. The Kozak MN14 heavy chain gene construct was digested with EcoRI and the heavy chain gene containing fragment was isolated. The IRES alpha-LA signal peptide MN14 light chain gene construct was also digested with EcoRI. The heavy chain gene was then **cloned** into the EcoRI site of IRES light chain construct. A multiple **cloning** site was then added to the LNCX retroviral backbone plasmid, to create LNC-MCS. Next a mutated double chain fragment was inserted into the alpha-LA expression retroviral backbone LN alpha-LA-Mertz-MCS. A WPRE element was then inserted into the gene construct. The plasmid Bluescript II SK+ WPRE-B11 was digested with BamHI and HincII to remove the WPRE element and the element was isolated. The vector created above was digested with BglII and HpaI. The WPRE fragment was ligated into the BglII and HpaI sites to create the final gene construct. Pseudotyped vector were made from the packaging cell lines for the vector alpha-LA MN14. MDBK cells (bovine kidney cells) were plated in a T25 flask and 10 to the power 6 colony forming units (CFUs) of vector in 3 ml media was incubated with the cells. The cell lines were grown to confluency in T25 flasks and the media was assayed for the presence of MN14. All of the MN14 produced was active and Western blotting showed that the heavy and light chains were produced at a 1:1 ratio.(151 pages)

L7 ANSWER 17 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-06350 BIOTECHDS
TITLE: Increasing polypeptide accumulation in plant protein storage tissue, by modifying polynucleotide encoding polypeptide so that it is uncleaved by proteases, transforming plant with expression cassette having **sequence**;
transgenic plant generation useful for recombinant protein, hormone and antibody production
AUTHOR: GRUIS D B; JUNG R
PATENT ASSIGNEE: PIONEER HI-BRED INT INC
PATENT INFO: US 2002108149 8 Aug 2002
APPLICATION INFO: US 2001-934066 21 Aug 2001
PRIORITY INFO: US 2001-934066 21 Aug 2001; US 2000-226804 21 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-066661 [06]
AN 2003-06350 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Increasing (M1) accumulation of polypeptide(s) in plant protein storage tissue, involves modifying nucleotide **sequence** encoding a polypeptide to produce modified nucleotide **sequence** that will not be cleaved by protease(s), and transforming expression cassette comprising modified **sequence** into plant to direct expression of the polypeptide in protein storage tissue produced by the plant.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a plant (I) that is genetically modified to reduce or eliminate the activity of one or more proteases in its protein storage tissue; (2) transformed seed obtained from (I); (3) producing (M2) one or more polypeptides in a plant protein storage tissue, involves modifying the nucleotide **sequence** encoding the polypeptide to produce a modified nucleotide **sequence** encoding a polypeptide that will not be cleaved by one or more proteases, and transforming an expression cassette comprising the modified nucleotide **sequence** into a plant where the expression cassette directs the expression of the polypeptide that will not be cleaved by one or more proteases in the

protein storage tissue of the plant; (4) a plant or polypeptide (II) produced by (M2); (5) preventing the proteolytic degradation of one or more polypeptides in plant protein storage tissues, involves performing the steps of (M1), encoding an amino acid **sequence** (S2) of 466 amino acids; (6) an isolated nucleic acid (III) molecule comprising a nucleotide **sequence** (S1) of 1560 base pairs (bp) defined in the specification, a nucleotide **sequence** having 60% **sequence** identity to (S1) which encodes a polypeptide having protease activity, a nucleotide **sequence** encoding an amino acid **sequence** (S2) of 466 amino acids, a nucleotide **sequence** encoding a fragment comprising 10 contiguous amino acids of (S2), a nucleotide **sequence** that hybridizes under stringent conditions to the complement of (S1) in 50% formamide, 1 M NaCl 1% sodium dodecyl sulfate at 37 degrees C and a wash in 15 mM NaCl, 1.5 mM trisodium citrate at 60 degrees C, or the complement of the above nucleotide **sequence**; and (7) an isolated polypeptide (IV) comprising an amino acid **sequence** of (S2), a fragment comprising 10 contiguous amino acids of (S2), **sequence** variant of (S2) having protease activity and encoded by a nucleotide **sequence** that hybridizes to complement of (S1) under stringent conditions or which has 60% **sequence** identity to (S1), or amino acid **sequence** encoded by (S1).

BIOTECHNOLOGY - Preferred Method: In (M1), the seed protease is vacuolar processing enzymes, aspartic proteases, papain-type proteases, subtilisins and serine endopeptidases, especially alpha-, beta-, gamma-, epsilon- vacuolar processing enzymes, and aspartic protease AP1 and AP2. Preferred Plant: In (I), the protease has an amino acid **sequence** chosen from (IV). (I) is dicot such as Arabidopsis, soybean, sunflower, canola, cotton and safflower, or is monocot such as maize, wheat, rice, barley, oats rye and millet. (I) is transformed with an expression cassette comprising a constitutive, tissue-preferred, leaf-specific, root-preferred or seed-preferred promoter, operably linked with a polypeptide of interest. (II) is a soy protein isolate.

USE - (M1) is useful for increasing accumulation of polypeptide(s) in plant protein storage tissue such as seed, tubers, roots, and leaves. (M1) is also useful for increasing the accumulation of one or more polypeptides of interest in seed, involves genetically modifying a plant to alter (i.e. increasing or decreasing) the activity of a protease in the seed of the plant by transforming the plant with an expression cassette comprising a promoter operably linked to the polypeptide of interest. The magnitude of the alteration of the activity of the protease is 2 fold. (M2) is useful for producing one or more polypeptides chosen from insulin, human growth hormone, pepsin, cellulases, pectinases, hemicellulases, phytases, hydrolases, esterases, peroxidases, fibrinogen, plasma proteins, serum albumin, factor IX, factor XIII, thrombin, protein C, xylanase, isoamylase, glucoamylase, alpha-amylase, lysozyme, catalase, beta-glucanase, beta-casein, lactase, urease, glucose isomerase, superoxide dismutase, pullulanase, invertase, streptavidin, avidin, alkaline phosphatase, aprotinin, beta-glucuronidase, protease inhibitors, pepsin, chymotrypsin, trypsin, papain, kinases, phosphatases, antibodies, deoxyribonucleases, ribonucleases, phospholipases, lipases, peptide hormones, green fluorescent protein, secreted antibodies, single chain antibodies, spider silk, soybean 2S albumin, glycinin and beta-conglycinin, in a plant protein storage tissue (all claimed). (M1) is useful in altering the nutritional or other qualities of seeds, and in producing useful proteins including pharmacological or industrial proteins. (III) is useful for isolating corresponding **sequences** from other organisms (especially other plants), constructing antisense constructions for other proteases, and in the sense orientation to suppress the expression of endogenous genes in plants.

ADVANTAGE - (M1) produces plants having altered composition such as increased nutritional value, **enhanced** food **processing** properties, and production of precursor molecules for industrial or pharmaceutical use. (M2) produces highly refined soy protein isolates.

EXAMPLE - Increased accumulation of Vacuolar-targeted polypeptides

of interest in the seed of Arabidopsis deficient for both beta-(vacuolar processing enzymes) VPE and epsilon-VPE was as follows. An expression cassette containing a functional fusion of the soybean alpha prime beta-conglycinin promoter (for seed-preferred expression), the coding **sequence** of the sporamin signal peptide (for import into the secretory pathway), and the coding **sequence** of the sporamin propeptide (containing vacuolar targeting information) was linked in frame with the bovine **sequence** encoding B-casein milk protein, and further was connected with the nopaline synthase 3' untranslated region (UTR) as a terminator. This expression cassette was **cloned** into a T-DNA vector also designed to express the NPTII selectable marker gene and transformed into wild-type Arabidopsis by vacuum infiltration of flowers. Selected transformants were crossed to Arabidopsis mutant lines that were either deficient in beta-VPE or epsilon-VPE protease activity or both protease activities. Progeny plants of the crosses were self-pollinated and plants of the next generation were screened for events that were hemizygous or homozygous for the dominant beta casein transgene and were homozygous for the recessive insertion mutant alleles of the beta-VPE and epsilon-VPE genes. These allele tests were conducted by **polymerase** chain reaction (**PCR**) with gene and transposon specific primers. Arabidopsis plants expressing the beta-casein gene alone or in combination with a deficiency in beta-VPE and epsilon-VPE activity were analyzed for the accumulation of B-casein in mature seed. The stable accumulation of beta-casein in mature seed of Arabidopsis deficient for beta-VPE, epsilon-VPE or both beta-VPE and epsilon-VPE, was two fold or more greater than that found in wild-type Arabidopsis. (22 pages)

L7 ANSWER 18 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-17098 BIOTECHDS
TITLE: New isolated regulatory region of the CCR5 gene, useful for identifying specific inhibitors, used e.g. to treat inflammation and infection by human immune deficiency virus; liposome vector-mediated gene transfer and expression in host cell for use in gene therapy
AUTHOR: GUIGNARD F; MURPHY P M; COMBADIERE C; TIFFANY H L
PATENT ASSIGNEE: US DEPT HEALTH and HUMAN SERVICES
PATENT INFO: US 6383746 7 May 2002
APPLICATION INFO: US 1997-177437 23 Oct 1997
PRIORITY INFO: US 1998-177437 21 Oct 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-442806 [47]
AN 2002-17098 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Isolated functional chemokine receptor 5 (CCR5) regulatory **sequence** (I) comprising nucleotides (nt) 36-1006 of a fully defined artificial 2916 base pair (bp) **sequence**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an antisense nucleic acid (II) comprising a triplex-forming oligonucleotide, or analog, that binds to the 927-1006 or 521-763 regions of (I), reducing its activity; and (2) identifying a compound that suppresses expression of CCR5.

WIDER DISCLOSURE - Disclosed is detection of chemokine receptor 5 (CCR5) mutants by **polymerase** chain reaction (**PCR**) and analysis of the amplicon by Southern blotting. Mutated **sequences** detected this way may then be **sequenced** and **cloned.**

BIOTECHNOLOGY - Preferred Materials: (I) may be linked to a **sequence** that encodes a heterologous protein, especially a cytotoxic agent, particularly one that is toxic only in presence of a pathogen-associated protein. (II) comprises: (i) any of four specific 20-mers comprising T and G only, e.g. TGT5GTTTGTTGTTGTG (5) or (ii) a **sequence** of 20-38 bases from either of two 38-mers, e.g. GT4GTGTGTGGT8GT5GTTGTTGTGTGGTG (9). **Sequence** (I) comprises two exons, separated by a 1.9 kb intron, and the promoter region contains

many features, including strong silencer and **enhancer** elements. Preferred **Process**: In (b), a test compound is incubated with (I) that is linked to a reporter gene (RG), then agents that inhibit regulatory activity of (I) are identified from ability to reduce RG expression. RG is particularly the LacZ, green fluorescent protein or chloramphenicol acetyltransferase (CAT) genes, or a reporter other than a gene, e.g. radioisotope, fluorophore, bio- or chemi-luminescent compound or metal chelator. The same method can be used to identify agents that modulate activity of a CCR5 promoter polymorphism. Preparation: The promoter region of the CCR5 gene was identified by amplifying parts of a 2.5 kb fragment containing the putative 5'-end of the gene, and **cloning** the parts upstream of the CAT gene in pCAT-basic. The recombinant plasmids formed were then tested for CAT expression.

ACTIVITY - Antiinflammatory; Antiasthmatic; Antiarthritic; Anti-HIV. No suitable data given.

MECHANISM OF ACTION - Inhibiting expression of CCR5 so as to inhibit activity of the proinflammatory proteins macrophage inflammatory protein-1alpha or beta, and RANTES, and inhibit entry of HIV into cells.

USE - (I), the promoter region of the CCR5 gene, is useful for identifying agents (A), particularly antisense triplex-forming oligonucleotides, that suppress expression of CCR5 (claimed). (A), optionally expressed from gene therapy vectors, are used to treat inflammation (e.g. asthma, arthritis or Crohn's disease) and to treat or prevent infection by human immune deficiency virus (HIV). (I) can also be used to direct expression of heterologous proteins selectively in myeloid and lymphoid cells, e.g. for ablation of such cells when the expressed protein is cytotoxic.

ADMINISTRATION - Antisense nucleic acids, or vectors that express them, are administered orally, by injection or topically, optionally in a liposome. No dose given.

EXAMPLE - No suitable example given.(26 pages)

L7 ANSWER 19 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-18621 BIOTECHDS
TITLE: Isolated truncated Apaf-1 polypeptide or its fragment for identifying an inhibitor or enhancer of Apaf-1 mediated caspase processing;
vector-mediated recombinant protein gene transfer and expression in host cell for use in cancer and autoimmune disease therapy
AUTHOR: ALNEMRI E S
PATENT ASSIGNEE: UNIV JEFFERSON THOMAS
PATENT INFO: AU 200227605 16 May 2002
APPLICATION INFO: AU 1999-27605 14 Jun 1999
PRIORITY INFO: AU 2002-27605 22 Mar 2002
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable AU
OTHER SOURCE: WPI: 2002-520375 [56]
AN 2002-18621 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated truncated (Apoptotic Protease Activating Factor 1) Apaf-1 polypeptide (I) or its fragment, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid molecule (II) encoding (I) or its variant; (2) an expression vector (III) comprising (I) operatively linked to a promoter; (3) a host cell (IV) transfected with (III); (4) an antisense nucleic acid molecule (IIa) comprising a nucleic acid **sequence** complementary to (II); (5) a gene delivery vehicle (V) comprising (II) operatively linked to a promoter; (6) identifying an inhibitor of apoptosis, by contacting a cell transfected with an inducible expression vector capable of expressing a self-oligomerizing and self-processing caspase with a candidate inhibitor, contacting the transfected cell with an inducer capable of self-oligomerizing caspase expression, and detecting the presence of large and small caspase processing activity or detecting cell viability; (7) inhibiting apoptosis in a cell, by

delivering a nucleic acid molecule encoding caspase-9 prodomain and maintaining the cell for the expression of the polypeptide; (8) inducing apoptosis in a cell, by delivering a nucleic acid molecule encoding self-oligomerizing caspase-9 polypeptide and maintaining the cell for the expression of the polypeptide; and (9) a gene delivery vehicle (Va) comprising a nucleic acid molecule encoding a self-oligomerizing caspase-9 polypeptide, operatively linked to a promoter.

BIOTECHNOLOGY - Preferred Protein: (I) is a human truncated Apaf-1 comprising a **sequence** of 1194 amino acids fully defined in the specification. (I) oligomerizes with a caspase selected from caspase-1 to caspase 13, or procaspase-9. The caspase is in vitro translated and labeled with a radioactive label, peptide tag, enzyme or biotin. Preferred **Sequence**: The promoter in (III) is inducible. Preferred Cell: (IV) is a bacterium, insect cell or a mammalian cell.Preferred Vehicle: In (V) or (Va), the vehicle is a retrovirus or adenovirus, and (II) is associated with a polycation. (V) or (Va) further comprises a ligand that binds a cell surface receptor.

ACTIVITY - Cytostatic; Immunosuppressive.

MECHANISM OF ACTION - Inhibitor or enhancer of Apaf-1 mediated caspase processing; inhibitor or inducer of apoptosis (claimed). No suitable data given.

USE - (I) is useful for identifying an inhibitor or enhancer of Apaf-1 mediated caspase processing, by contacting a sample containing (I) and one or more caspases with a candidate inhibitor or candidate enhancer, detecting the presence of large and small caspase subunits, and determining the level of caspase processing activity, where a decrease in processing indicates the presence of a caspase processing inhibitor, and where an increase in processing indicates the presence of a caspase **processing enhancer.** (IV) is useful for identifying an inhibitor or enhancer of Apaf-1 mediated caspase processing, by contacting a cell transfected with (III) with a candidate inhibitor or candidate enhancer, and detecting the presence of large and small caspase subunits, as described above. (II) is useful for inducing apoptosis in a cell, by delivering (II) to a cell, by injecting (II) into the cell or administering (II) to the circulatory system of a warm-blooded mammal in which the cell is located. (V) or (Va) is useful for treating cancer and autoimmune diseases, by internalizing (V) or (Va) by tumor cells. (III) is useful for identifying an inhibitor of Apaf-1 mediated caspase processing or apoptosis processing, by contacting a cell transfected with (III) with a candidate inhibitor, contacting the transfected cell with an inducer capable of inducing truncated Apaf-1 expression, and detecting the presence of large and small caspase subunits and thus determining the level of caspase processing activity (claimed).

EXAMPLE - To express the caspases or truncated Apaf-1 in mammalian cells and assay their apoptotic activity, truncated Apaf-1 or caspase was amplified with T7-tag primer and reverse primer using the pET28a construct as template and subcloned into the mammalian double expression vector pRSC-LacZ (MacFarlane et al., J. Biol. Chemical 272:25417-25420, 1997; Tsang et al., Bio/Technology, 22:68, 1997) or as previously described (Li et al., Cell 91:479-489, 1997; Srinivasula et al., J. Biol. Chemical 272:18452-18545, 1997). Complementary deoxyribonucleic acids (cDNA)s encoding truncated or mutated Apaf-1 variants were generated by **polymerase** chain reaction (**PCR**) and subcloned into the bacterial expression vectors pET-28a or pET-21b. This vector allows the expression of lacZ under the Rous Sarcoma virus promoter, and the test cDNA under the CMV promoter. To assay for apoptosis, MCF-7 or 293 cells were transfected with the pRSC-LacZ constructs in the presence or absence of different apoptosis-inhibitors. 30 hours after transfection cells were stained with beta-galactosidase and examined for morphological signs of apoptosis. The percentage of round blue apoptotic cells (mean +/- SD) were determined by phase contrast microscopy and then represented as a function of total blue cells under each condition (n at least 3). Test details are described but no results given.(77 pages)

ACCESSION NUMBER: 2002301224 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11919186
TITLE: The estrogen-responsive B box protein: a novel regulator of keratinocyte differentiation.
AUTHOR: Beer Hans-Dietmar; Munding Christine; Dubois Nicole; Mamie Celine; Hohl Daniel; Werner Sabine
CORPORATE SOURCE: Institute of Cell Biology, Department of Biology, ETH Zurich, CH-8093 Zurich, Switzerland.. Hans-Dietmar.beer@cell.biol.ethz.ch
SOURCE: Journal of biological chemistry, (2002 Jun 7) 277 (23) 20740-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF449496
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020604
Last Updated on STN: 20030105
Entered Medline: 20020712

AB Keratinocyte growth factor (KGF) regulates proliferation, differentiation, migration, and survival of different types of epithelial cells, including keratinocytes of the skin. To gain insight into the mechanisms underlying these multiple functions, we searched for KGF- regulated genes in keratinocytes. Using the differential display reverse transcriptase-**PCR** technology, we identified the gene encoding the estrogen-responsive B box protein (EBBP) which has as yet not been functionally characterized. The full-length murine and human EBBP cDNAs were **cloned** and fully **sequenced.** They were shown to encode 75-kDa proteins, which are mainly localized in the cytoplasm of keratinocytes in vitro and in vivo. In vivo, EBBP was found at high levels in the KGF- and epidermal growth factor-responsive basal keratinocytes of human skin, but the expression was down-regulated in the hyperthickened epithelium of skin wounds. Stable overexpression of EBBP in HaCaT keratinocytes did not affect the proliferation rate of the transfected cells, but **enhanced** the early differentiation **process.** These results suggest that the presence of EBBP in basal keratinocytes is important for the differentiation capacity of these cells, and that down-regulation of EBBP expression in a hyperproliferative epithelium is required to maintain the cells in a non-differentiated stage.

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L7 ANSWER 21 OF 43 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2002241198 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11805086
TITLE: Elucidation of an archaeal replication protein network to generate enhanced **PCR** enzymes.
AUTHOR: Motz Michael; Kober Ingo; Girardot Charles; Loeser Eva; Bauer Ulrike; Albers Michael; Moeckel Gerd; Minch Eric; Voss Hartmut; Kilger Christian; Koegl Manfred

CORPORATE SOURCE: Exploratory Research, LION Bioscience Ktiengesellschaft, D-69120 Heidelberg, Germany.
SOURCE: Journal of biological chemistry, (2002 May 3) 277 (18) 16179-88.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020430
Last Updated on STN: 20030105
Entered Medline: 20020702

AB Thermostable DNA polymerases are an important tool in molecular biology. To exploit the archaeal repertoire of proteins involved in DNA replication for use in **PCR**, we elucidated the network of proteins implicated in this process in Archaeoglobus fulgidus. To this end, we performed extensive yeast two-hybrid screens using putative archaeal replication factors as starting points. This approach yielded a protein network involving 30 proteins potentially implicated in archaeal DNA replication including several novel factors. Based on these results, we were able to improve **PCR** reactions catalyzed by archaeal DNA polymerases by supplementing the reaction with predicted **polymerase** co-factors. In this approach we concentrated on the archaeal proliferating cell nuclear antigen (PCNA) homologue. This protein is known to encircle DNA as a ring in eukaryotes, tethering other proteins to DNA. Indeed, addition of A. fulgidus PCNA resulted in marked stimulation of **PCR** product generation. The PCNA-binding domain was determined, and a hybrid DNA **polymerase** was constructed by grafting this domain onto the classical **PCR** enzyme from Thermus aquaticus, Taq DNA **polymerase.** Addition of PCNA to **PCR** reactions catalyzed by the fusion protein greatly stimulated product generation, most likely by tethering the enzyme to DNA. This sliding clamp-induced increase of **PCR** performance implies a promising novel micromechanical principle for the development of **PCR** enzymes with **enhanced processivity.**

L7 ANSWER 22 OF 43 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2002348386 EMBASE
TITLE: Increased expression of inducible nitric oxide synthase for human buccal squamous-cell carcinomas: Immunohistochemical, reverse transcription-**polymerase** chain reaction (RT-**PCR**) and in situ RT-**PCR** studies.
AUTHOR: Chen Y.-K.; Hsue S.-S.; Lin L.-M.
CORPORATE SOURCE: Dr. L.-M. Lin, Oral Pathology Department, School of Dentistry, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung, Taiwan,Province of China. k0285@ms22.hinet.net
SOURCE: Head and Neck, (1 Oct 2002) 24/10 (925-932).
Refs: 24
ISSN: 1043-3074 CODEN: HEANEE
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Background. The inducible nitric oxide synthase (iNOS) is involved primarily in inflammatory and carcinogenesis **processes.** An **enhanced** expression of iNOS at the protein level has been reported previously for human oral squamous cell carcinoma; however, the expression of iNOS at the mRNA level has not yet been demonstrated. Furthermore, no studies have addressed whether iNOS expression at mRNA level correlates with cervical lymph node metastasis. Methods. Specimens of the squamous

cell carcinoma of the buccal mucosa obtained from tissue samples of surgically resected tumors from 25 male patients were evaluated with immunohistochemical assessment of iNOS protein and IS-RT-**PCR**, as well as RT-**PCR** for iNOS mRNA. We also analyzed the iNOS expression status with clinical parameters to determine whether it had any prognostic significance in this homogenous population. Results. Inducible NOS protein (16 of 25, 64%) and mRNA (13 of 25, 53%) activities were detected for the oral carcinoma specimens examined in this study. Cytoplasmic and/or nuclear stainings were observed in the specimens of both well-differentiated SCC (10 of 15, 67%), and moderately to poorly differentiated SCC (6 of 10, 60%). The cellular location of iNOS mRNA was noted to be consistent with the finding using immunohistochemical technique (cytoplasm and/or nuclei stainings of the tumor islands). Using in situ RT-**PCR**, iNOS mRNA activity was detected in nine specimens of well-differentiated squamous cell carcinoma (9 of 15, 60%) and four specimens of moderately to poorly differentiated squamous cell carcinoma (4 of 10, 40%). With RT-**PCR**, an electrophoretic band corresponding to a 907-bp **PCR** product was observed for nine specimens of well-differentiated squamous cell carcinoma (9 of 15, 60%) and four specimens of moderately to poorly differentiated squamous-cell carcinoma (4 of 10, 40%). Neither iNOS protein nor mRNA was noticed in the samples of normal buccal mucosa or in the negative control samples. There was a significant relationship between iNOS expression (at both protein and mRNA levels) and whether patients had nodal disease. Conclusions. We have demonstrated an enhanced expression of iNOS protein and mRNA in a number of human buccal carcinomas compared with normal buccal mucosa. Such an observation suggests that the iNOS protein and mRNA expression is chiefly derived from a subset of oral cancerous tissues. Our observation also indicates that iNOS expression correlates with cervical lymph node metastasis in oral squamous cell carcinomas. Therefore, it may also indicate that a subset of oral cancers with greater metastatic potential, as evidenced by increased expression iNOS protein and mRNA, is identified.

L7 ANSWER 23 OF 43 EMBASE
on STN DUPLICATE 7

ACCESSION NUMBER: 2002323246 EMBASE
TITLE: Detection of rodent Helicobacter spp. by use of fluorogenic nuclease **polymerase** chain reaction assays.
AUTHOR: Drazenovich N.L.; Franklin C.L.; Livingston R.S.; Besselsen D.G.
CORPORATE SOURCE: Dr. D.G. Besselsen, Department of University Animal Care, University of Arizona, Tucson, AZ 85721-0101, United States
SOURCE: Comparative Medicine, (2002) 52/4 (347-353).
Refs: 33
ISSN: 0023-6764 CODEN: COMEFT
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Polymerase** chain reaction (**PCR**) analysis is the standard method for detection of Helicobacter spp. infections in laboratory rodents, with H. hepaticus, H. bilis, and H. typhlonius considered primary pathogens. Fluorogenic nuclease **PCR** assays that detect all known rodent Helicobacter spp., or that specifically detect H. hepaticus, H. bilis, or H. typhlonius were developed to eliminate post-**PCR processing**, **enhance** specificity, and provide quantitative data on starting template concentration. Each fluorogenic **PCR** assay detected a minimum of 10 copies of target template, had comparable or greater sensitivity when compared directly with corollary gel detection **PCR** assays, and detected only targeted species when numerous Helicobacter spp. and other enteric bacteria were analyzed. Fluorogenic nuclease **PCR** analysis of fecal DNA samples obtained from numerous laboratory mice

sources detected all samples with positive results by use of Helicobacter spp., H. hepaticus, H. bilis, and/or H. typhlonius gel detection **PCR** analysis, except for one sample that had positive results by H. typhlonius gel detection **PCR** but negative results by H. typhlonius fluorogenic nuclease **PCR** analysis. Among fecal DNA samples that were Helicobacter spp. negative by use of all gel detection **PCR** assays, the fluorogenic nuclease **PCR** assays detected target template in only one sample that was positive by use of the Helicobacter spp. and the H. bilis fluorogenic nuclease **PCR** assays. In conclusion, fluorogenic nuclease **PCR** assays provide sensitive, specific, and high-throughput diagnostic assays for detection of Helicobacter spp., H. hepaticus, H. bilis, and H. typhlonius in laboratory rodents, and the quantitative data generated by these assays make them potentially useful for bacterial load determination.

L7 ANSWER 24 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:903241 CAPLUS
DOCUMENT NUMBER: 137:88937
TITLE: **Polymerase** chain reaction-mediated **mutagenesis** in **sequence** resistant to homogeneous amplification
AUTHOR(S): Nazar, Ross N.; Abeyrathne, P. D.; Intine, Robert V. A.
CORPORATE SOURCE: Department of Molecular Biology and Genetics, University of Guelph, ON, Can.
SOURCE: Methods in Molecular Biology (Totowa, NJ, United States) (2002), 182(In Vitro Mutagenesis Protocols (2nd Edition)), 117-126
CODEN: MMBIED; ISSN: 1064-3745
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The methods for using **polymerase** chain reaction (**PCR**) as a tool for homogeneous DNA amplification are described. One of these methods is called plasmid-enhanced **PCR**-mediated **mutagenesis**, which reduces the artifacts caused by the recombination between complementary **sequence** elements during DNA amplification. In PEP **mutagenesis**, the mutant DNA is amplified as two halves of the final **sequence** and simply joined by blunt ligation. For a deletion, the targeted **sequence** is simply omitted and, for base substitutions or insertions, the mutant **sequence** is incorporated into one of the primers. The efficiency and orientation of the ligation **process** is **enhanced** or controlled by **sequence**-specific overlapping interactions with the plasmid using compatible restriction sites.
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 25 OF 43 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2002283106 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12022389
TITLE: Detection of rodent coronaviruses by use of fluorogenic reverse transcriptase-**polymerase** chain reaction analysis.
AUTHOR: Besselsen David G; Wagner April M; Loganbill Jessie K
CORPORATE SOURCE: Department of University Animal Care, The University of Arizona, Tucson 85721-0101, USA.
CONTRACT NUMBER: R01 RR14072 (NCRR)
SOURCE: Comparative medicine, (2002 Apr) 52 (2) 111-6.
Journal code: 100900466. ISSN: 1532-0820.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20020528
Last Updated on STN: 20030202
Entered Medline: 20030131

AB Reverse transcriptase-**polymerase** chain reaction (RT-**PCR**) assays have proved useful for the detection of mouse hepatitis virus (MHV) and rat coronavirus (RCV) in acutely infected animals and contaminated biomaterials. Fluorogenic nuclease RT-**PCR** assays combine RT-**PCR** with an internal fluorogenic hybridization probe, thereby eliminating post-**PCR processing** and potentially **enhancing** specificity. Consequently, a fluorogenic nuclease RT-**PCR** assay specific for rodent coronaviruses was developed. Primer and probe **sequences** were selected from the viral genome segment that encodes the membrane (M) protein that is highly conserved among rodent coronaviruses. Use of the fluorogenic nuclease RT-**PCR** detected all strains of MHV and RCV that were evaluated, but did not detect other RNA viruses that naturally infect rodents. Use of the assay detected as little as two femtograms of in vitro transcribed RNA generated from **cloned** amplicon, and when compared directly with mouse antibody production tests, had similar sensitivity at detecting MHV-A59 in infected cell culture lysates. Finally, use of the assay detected coronavirus RNA in tissues, cage swipes, and feces obtained from mice experimentally infected with MHV, and in tissues and cage swipes obtained from rats naturally infected with RCV. These results indicate that the fluorogenic nuclease RT-**PCR** assay should provide a potentially high-throughput, **PCR**-based method to detect rodent coronaviruses in infected rodents and contaminated biological materials.

L7 ANSWER 26 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-07115 BIOTECHDS
TITLE: Production of platelet derived growth factor (PDGF) comprises expression in the milk of a non-human transgenic animal; recombinant protein production by vector expression in cell and transgenic animal useful in disease therapy
AUTHOR: ECHELARD Y; MEADE H; EICHNER W; SOMMERMEYER K
PATENT ASSIGNEE: GENZYME TRANSGENICS CORP
PATENT INFO: WO 2001098520 27 Dec 2001
APPLICATION INFO: WO 2000-US41044 19 Jun 2000
PRIORITY INFO: US 2000-212406 19 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-083329 [11]
AN 2002-07115 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Production (M1) of platelet derived growth factor (PDGF) comprises a transgenic mammal whose somatic and germ cells comprise a nucleic acid **sequence** (I) encoding PDGF, operably linked to a promoter directing expression into mammalian gland epithelial cells, and obtaining the milk from the transgenic mammal where at least 30% of the PDGF in the milk is as a dimer.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) producing (M2) a transgenic mammal capable of expressing an active PDGF molecule in it's milk. This comprises introducing into a cell (I) encoding PDGF chains operably linked to a promoter which directs expression in mammary epithelial cells. The cell gives rise to a transgenic mammal which expresses PDGF in its milk, where at least 30% of the PDGF is present in the milk in its active form; (2) a milk preparation obtained from a transgenic mammal whose genome contains (I) encoding at least one PDGF chain operably linked to a promoter which directs expression in mammary epithelial cells, where the PDGF chain is expressed in the mammary epithelial cells of the transgenic animal, and where at least 30% of the PDGF in the milk is present as a dimer; and (3) an isolated nucleic acid (I) comprising a nucleic acid **sequence** encoding a biologically active PDGF, or homologue operatively linked to a regulatory **sequence** capable of directing the expression of PDGF

in the mammary gland of non-human transgenic animals.

WIDER DISCLOSURE - Also disclosed is a pharmaceutical composition comprising PDGF, a transgenic PDGF preparation, a transgenic animal which expresses PDGF, production of an analog of PDGF and a method of providing/administering transgenically produced PDGF to a subject in need of PDGF.

BIOTECHNOLOGY - Preparation: PDGF was produced by standard recombinant procedures. Preferred Methods: In M1 the nucleic acid **sequence** (I) can encode a PDGF A chain or a PDGF B chain, under the control of the same promoter, where at least 30% of the PDGF in the milk is as a PDGF-AA or PDGF-BB homodimer. (I) when encoding the PDGF A chain is operably linked to a different promoter than (I) when encoding the PDGF B chain. The transgenic mammal can comprise (I) encoding a PDGF A chain and (I) encoding a PDGF B chain. In M2 to produce a transgenic mammal, the somatic cell or the nucleus of the somatic cell is introduced into the oocyte. (I) encoding a PDGF A chain and (I) encoding a PDGF B chain operably linked to a promoter which directs expression in mammary epithelial cells, is introduced into this cell. M2 also comprises providing a cell from a transgenic mammal whose germ and somatic cells comprise (I) encoding a PDGF-A chain operably linked to a promoter directing expression in mammary epithelial cells, and introducing into a cell (I) encoding a PDGF-B chain as above, and allowing the cell to give rise to a transgenic animal. Preferred Milk Preparation: In the milk preparation, the PDGF chain is either the PDGF-A or PDGF-B chain, and at least 30% of the PDGF is present in the milk as a PDGF-AA or PDGF-BB homodimer. Also the genome of the transgenic animal can comprise (I) encoding a PDGF- A chain and (I) encoding a PDGF-B chain under the control of a promoter which directs expression in mammary epithelial cells. The milk preparation then comprises PDGF-AB heterodimer at 30% of the PDGF present in the milk. The milk preparation comprises at least 1mg/ml PDGF. The transgenically produced PDGF is human and differs in its glycosylation pattern from PDGF occurring naturally or recombinantly isolated. Preferred Mammal: The transgenic mammal is a goat. Preferred Nucleic Acid: (I) is an isolated nucleic acid comprising a **sequence** encoding a biologically active PDGF, or homologue, operatively linked to a regulatory **sequence** capable of directing the expression of PDGF in the mammary gland of non-human transgenic animals. (I) can encode a PDGF-A chain and a PDGF-B chain, or a PDGF- B chain. (I) is mono- or dicistronic, preferably dicistronic, and comprises the expression cassette BC701 or BC734, whose fully defined **sequence** is given in the specification. Preferred Promoters: The mammary gland specific promoter can be a caesin promoter, beta lactoglobulin promoter, whey acid protein promoter or lactalbumin promoter.

USE - For production of PDGF (claimed). Pharmaceutical compositions can be obtained from this milk and can be used to stimulate or **enhance** the wound healing **process**, in particular diabetic foot ulcers, decubitis ulcers and venous stasis ulcers. Transgenic PDGF(II) can also be used in the treatment of periodontal regeneration, stimulation of bone formation, ophthalmic diseases or healing of prosthetic vascular grafts. (II) can also be used for non-medical applications, e.g., as a supplement for cell culture media or as a component of diagnostic kits.

EXAMPLE - Two expression cassettes BC701 (PDGF-B) and BC734 (PDGF-A-IRESG-PDGF-B) were constructed using **sequences** isolated from pSBC-PDGF-A/-G-B (III) (described in patent US5665567). To create BC701, (III) was partially cut with HindIII and ligated to the self-annealing cohesive linker HINXHO, **sequence** AGCTCTCGAG, thereby destroying the HindIII site and creating an XhoI site. Plasmid pAB21 which had one copy of HINXHO integrated into the HindIII site located at the 3' end of the PDGF-B gene was identified using restriction mapping. This plasmid was then partially cut with EcoRI and ligated to the self-annealing cohesive ECOXHO, **sequence** AATTCTCGAG. Integration of this linker created an XhoI site. Plasmid pAB23 which had a copy of ECOXHO integrated into the EcoRI site at the 5' end of the

PDGF-B gene was identified using restriction mapping . Complete digestion with XhoI gave a 750 base pair fragment containing the full **sequence** of the PDGF-B190 gene (described in EP658198). This codes for a translation product identical to fully processed PDGF-BB. A stop codon was introduced at position 191 of the PDGF-B precursor protein, so that the carboxy terminal part of the PDGF-B molecule, which is responsible for the retention of incompletely processed forms is not expressed. This fragment, whose fully defined **sequence** is given in the specification, was isolated and **cloned** into the XhoI site of the mammary gland expression vector pBC450 to create PDGF-B expression cassette pBC701. This expression vector also contains **sequences** coding for the chicken beta-globin insulator **sequence** as well as the goat beta-caesin promoter, where these **sequences** are fully defined in the specification. PDGF-A-IRESG-PDGF-B was created by digesting pAB21 with Not1, filling in and self-ligating the ends, resulting in destroying the Not1 site in the IRES/G **sequence** of the resultant plasmid pAB2. This plasmid was cut with EcoRI and ligated to the self annealing cohesive linker ECONOXHO, **sequence** AATTGCTCGAGC, creating an XhoI site. The plasmid pAB33 with one copy of ECONOXHO integrated into the EcoRI site located at the 5' end of the PDGF-A gene was identified using restriction mapping. Complete digestion with XhoI gave a 2kb fragment containing the full **sequence** of the PDGF-A gene as well as PDGF-B190, both genes separated by the IRESG **sequences.** This 2kb fragment was isolated and ligated into the mammary gland expression vector pBC450 to create the expression cassette pBC734. The inserts of both transgenes, pBC701 and pBC734, were **sequenced** and verified prior to microinjection. The fully defined **sequence** of the pBC734 insert (PDGFB-IRESG-PDGFA) is given in the specification. 100 microg of expression cassette DNA was separated from the vector backbone by digestion with Not1 and prepared for microinjection. CD1 female mice were superovulated and the fertilized ova were retrieved from the oviduct. Male pronuclei were then microinjected with DNA. The microinjected embryos were either cultured overnight in CZB media according to Chartot et al. (Journal of Reproduction and Fertility, vol 86 (1989), 679-688), or transferred immediately into the oviduct of pseudopregnant recipient CD1 female mice. Twenty to thirty 2-cell or forty to fifty one cell embryos were transferred to each recipient female and allowed to continue to term. Founder animals were detected by **polymerase** chain reaction (**PCR**) of genomic DNA from tail tissue. Twenty-three BC701 founder animals were identified and mated. First generation offspring from 18 transgenic lines were mated and milk was collected. Female mice were allowed to deliver their pups naturally and were milked twice between days 6 and 12 post partum. Mice were induced to lactate using an intraperitoneal injection of 5 i.U. Oxytocin, followed by a 1-5 minute waiting time. Milk was collected by a suction and collection system and continued until at least 150 microl of milk had been obtained. PDGF was isolated from the milk by a clarification method comprising: (a) diluting milk 2:1 with 2.0 M Arginine-HCl pH 5.5; (b) spinning diluted sample for 20 minutes at 4-8 degreesC; (c) cooling sample for 5 mins to allow fat to solidify; (d) removing fat pad; and (e) decanting supernatant. The sample was further purified by affinity purification. Milk samples were analyzed using PDGF-B Western blot and activity assays. PDGF-B is expressed at 2-4 mg/ml in the milk of the founder female 647 and to a level of 0.5-1 mg/ml in the milk of female 484. (59 pages)

L7 ANSWER 27 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-05699 BIOTECHDS
TITLE: Protein for nucleic acid modification, consists of a heterologous **sequence**-non-specific double-stranded nucleic-acid-binding domain joined to a heterologous catalytic nucleic-acid-modifying domain;
plasmid pYW1-mediated catalytic DNA-modifying domain and ds DNA-binding domain fusion protein gene transfer, expression in host cell for **polymerase** chain

reaction

AUTHOR: WANG Y
PATENT ASSIGNEE: MJ BIOWORKS INC
PATENT INFO: WO 2001092501 6 Dec 2001
APPLICATION INFO: WO 2000-US17492 26 May 2000
PRIORITY INFO: US 2000-640958 16 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-075467 [10]
AN 2002-05699 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A protein (I) consisting of heterologous **sequence** -non-specific double-stranded nucleic-acid-binding domain (D1) joined to heterologous catalytic nucleic-acid-modifying domain (D2) having **processive** nature (presence of D1 **enhances** **processive** nature of D2 or stabilizes double-stranded conformation of a nucleic acid by at least 1degreesC, when compared to identical protein not having D1 joined to it), is new.

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques. Preferred Protein: In (I), D2 has thermally stable **polymerase**, RNA **polymerase**, reverse transcriptase, methylase, 3' or 5' exonuclease, gyrase or topoisomerase activity. D2 comprises a Thermus or Pyrococcus **polymerase** domain. D1 specifically binds to polyclonal antibodies generated against Sac7d or Sso7d, or binds to polyclonal antibodies generated against a PCNA homolog of P. furiosus. D1 is Sso7d or the PCNA homolog of P. furiosus. D1 contains a 50 amino acid subsequence containing 50%, preferably 75% amino acid similarity to Sso7d.

USE - (I) is useful for modifying a nucleic acid in an aqueous solution by contacting the nucleic acid with (I) or an aqueous solution containing (I), where the solution is at a temperature and of a composition that permits the binding domain to bind to the nucleic acid and the enzyme to function in a catalytic manner, and permitting the catalytic domain to modify the nucleic acid in the solution. (I) is useful for amplifying a subsequence of a target nucleic acid using a **polymerase** chain reaction (**PCR**) by contacting the target in an aqueous solution with (I), where the aqueous solution is of a composition that permits the binding domain to bind to the target nucleic acid and the **polymerase** domain to extend the primer that is hybridized to the target, and incubating the solution using the **PCR** temperature profile that amplifies the subsequence (claimed).

ADVANTAGE - D1 increases the processivity and/or efficiency of a modifying enzyme to which it is joined by stabilizing the enzyme-nucleic acid complex, and stabilizes the double-stranded conformation of a nucleic acid and increases the efficiency of the catalytic domain that requires a double-stranded substrate. (I) exhibits higher processivity while retaining error-correcting activity and provides both sensitivity and fidelity in amplification reaction.

EXAMPLE - Construction of Sso7d-deltaTaq fusion protein was as follows: A **polymerase** protein possessing **enhanced** **processivity** was constructed, in which the **sequence** -non-specific double-stranded nucleic acid binding protein Sso7d was fused to the Thermus aquaticus PolI DNA **polymerase** (a family A **polymerase** known as Taq DNA **polymerase**) that was deleted at the N terminus by 289 amino acids (deltaTaq). Based on the published amino acid **sequence** of Sso7d, seven oligonucleotides were used in constructing a synthetic gene encoding Sso7d. The oligonucleotides were annealed and ligated using T4 DNA ligase. The final ligated product was used as the template in a **polymerase** chain reaction (**PCR**) using two terminal oligonucleotides as primers to amplify the full-length gene. By design, the resulting **PCR** fragment contained a unique EcoRI site at the 5' terminus, and a unique BstXI site at the 3' terminus. In addition to encoding the Sso7d protein, the **PCR** fragment also encoded a peptide linker with the amino acid **sequence** of GGVT positioned at the C terminus of the Sso7d

protein. The synthetic gene encoding Sso7d was then used to generate a fusion protein in which Sso7d replaces the first 289 amino acid of Taq. The fragment encoding Sso7d was subcloned into a plasmid encoding Taq **polymerase** to generate the fusion protein, as follows. The DNA fragment containing the synthetic Sso7d gene was digested with restriction endonucleases EcoRI and BstXI, and ligated into the corresponding sites of a plasmid encoding Taq. As the result, the region that encoded the first 289 amino acid of Taq was replaced by the synthetic gene of Sso7d. This plasmid (pYW1) allowed the expression of a single polypeptide containing Sso7d fused to the N terminus of deltaTaq through a synthetic linker composed of GGVT.(65 pages)

L7 ANSWER 28 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-05054 BIOTECHDS
TITLE: New proteorhodopsin gene for use in a light-driven energy generator, comprises an isolated DNA **sequence** encoding a proteorhodopsin protein;
useful for drug delivery, biocatalyst reactor, fuel cell, molecular switch, molecular electronics, increased recombinant protein yield and gene expression modulation
AUTHOR: DELONG E F; BEJA O
PATENT ASSIGNEE: MONTEREY BAY AQUARIUM RES INST
PATENT INFO: WO 2001083701 8 Nov 2001
APPLICATION INFO: WO 2000-US14394 3 May 2000
PRIORITY INFO: US 2000-201602 3 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114151 [15]
AN 2002-05054 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A proteorhodopsin gene (I), comprising an isolated DNA **sequence** for encoding a proteorhodopsin protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) retrieving (I) comprising: (a) providing a sample of naturally occurring bacteria; (b) extracting a genomic fragment of the sample of naturally occurring bacteria; and (c) amplifying (I) from the genomic fragment using a **polymerase** chain reaction; (2) a light-driven energy generator (II), comprising: (i) a proteorhodopsin protein; (ii) a host to correctly fold the proteorhodopsin protein in the host, creating an integrated proteorhodopsin protein; and (iii) a source of retinal to bind covalently to the integrated proteorhodopsin protein, thereby creating a light absorbing pigment; (3) making a light driven energy generator, comprising: (a) providing a proteorhodopsin protein; (b) providing a host to correctly fold the proteorhodopsin protein in the host, creating an integrated proteorhodopsin protein; and (c) providing a source of retinal to bind covalently to the integrated proteorhodopsin protein, creating a light absorbing pigment; (4) a **polymerase** chain reaction (**PCR**) apparatus for amplifying a proteorhodopsin gene from DNA samples of naturally occurring microbial populations using **PCR**, comprising oligodeoxynucleotide primers with a Watson-Crick base pair complementarity to 5' and 3' ends of the proteorhodopsin gene; and (5) designing **PCR** primers, comprising: (a) determining a DNA **sequence** of a proteorhodopsin gene; and (b) based on the determined DNA **sequence**, designing oligodeoxynucleotide primers with a Watson-Crick base pair complementarity to the 5' and 3' ends of the proteorhodopsin gene.

WIDER DISCLOSURE - The following are disclosed: (1) proteorhodopsin-specific **polymerase** chain reaction primers to amplify a proteorhodopsin gene from DNA samples of marine bacteria; (2) proteorhodopsin protein **sequences** retrieved from samples of naturally occurring members of the domain bacteria; and (3) producing and obtaining variants of proteorhodopsin genes and proteins.

BIOTECHNOLOGY - Isolation: (I) is prepared by: (a) providing a sample of naturally occurring bacteria (e.g. marine proteobacteria or SAR86 bacteria); (b) extracting a genomic fragment of the sample of

naturally occurring bacterial; and (c) amplifying the proteorhodopsin gene from the genomic fragment using **PCR.** An expression vector containing the proteorhodopsin gene is created. The naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library or bacterial artificial chromosome library, or from any one of the 30 **clones**, given in the specification, that are BAC31A8, BAC40E8, BAC41B4, BAC64A5, HOT0ml, HOT75ml, HOT75ml3, HOT75m4, HOT75m8, MB0ml, MB0m2, MB20m2, MB20m5, MB20ml12, MB40ml, MB40m5, MB40ml12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6, and PALE7. Preferred Gene: (I) is amplified from a genomic fragment by **PCR** using primers of **sequence** (P1) and (P2). Preferred Method: Designing primers further involves using the oligodeoxynucleotide primers to amplify the proteorhodopsin gene from DNA samples of naturally occurring microbial populations by **PCR**, and **cloning** the amplified proteorhodopsin gene into an expression vector. accatgggtaaattattactgatatagg (P1) agcattagaagattctttaacagc (P2)

USE - (I) is useful in light-driven energy generators. A proteorhodopsin system is useful in many industrial and technological applications, for use in targeted drug delivery, has primary and secondary energy generators for biocatalytic reactors, fuel cells and nano-machines, as well as uses in molecular switching or data storage devices. Proteorhodopsin is useful for a **process** to **enhance** yield or increase the potential of recombinant protein production or convert the light-induced membrane potential into cellular signals, including the modulation of gene expression.

ADVANTAGE - The system is not restricted to operate in halophilic arachaea and could therefore be functionally expressed in Escherichia coli and other bacteria. It provides for a fast and cheap production method that allows for mass production of functionally active proteorhodopsin.

EXAMPLE - None given. (460 pages)

L7 ANSWER 29 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:246613 CAPLUS
DOCUMENT NUMBER: 134:248837
TITLE: Archaeoglobus fulgidus exonuclease III with 3'-exonuclease activity and its use in high fidelity amplification of DNA
INVENTOR(S): Ankenbauer, Waltraud; Franck, Laue; Sobek, Harald; Michael, Greif
PATENT ASSIGNEE(S): Roche Diagnostics G.m.b.H., Germany
SOURCE: Eur. Pat. Appl., 29 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1088891	A1	20010404	EP 1999-119268	19990928
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
WO 2001023583	A2	20010405	WO 2000-EP9423	20000927
WO 2001023583	A3	20011025		
W: AU, CA, CN, IL, JP, NO, NZ, RU, SG, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1144653	A2	20011017	EP 2000-969312	20000927
EP 1144653	A3	20020213		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2003510084	T2	20030318	JP 2001-526965	20000927
NO 2001002561	A	20010718	NO 2001-2561	20010525

PRIORITY APPLN. INFO.: EP 1999-119268 A 19990928
WO 2000-EP9423 W 20000927

AB The present invention provides **cloning** and expression of Archaeoglobus fulgidus exonuclease III with 3'-5'-exonuclease activity. According to the present invention a thermostable enzyme exhibiting 3'-exonuclease activity but no **polymerase** activity is provided whereas this enzyme **enhances** fidelity of an amplification **process** when added to a second enzyme with **polymerase** activity.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 30 OF 43 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 9

ACCESSION NUMBER: 2001334993 EMBASE
TITLE: Detection of rodent parvoviruses by use of fluorogenic nuclease **polymerase** chain reaction assays.
AUTHOR: Redig A.J.; Besselsen D.G.
CORPORATE SOURCE: Dr. D.G. Besselsen, University of Arizona, University Animal Care Building 101, Central Animal Facility, 1127 East Lowell Street, Tucson, AZ 85721-0101, United States
SOURCE: Comparative Medicine, (2001) 51/4 (326-331).
Refs: 25
ISSN: 0023-6764 CODEN: COMEFT
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Polymerase** chain reaction (**PCR**) assays have proven useful for detection of rodent parvoviruses in animals and contaminated biological materials. Fluorogenic nuclease **PCR** assays combine **PCR** with an internal fluorogenic hybridization probe, eliminating post-**PCR processing** and potentially **enhancing** specificity. Consequently, three fluorogenic nuclease **PCR** assays were developed, one that detects all rodent parvoviruses, one that specifically detects minute virus of mice (MVM), and one that specifically detects mouse parvovirus 1 (MPV) and hamster parvovirus (HaPV). When rodent parvoviruses and other rodent DNA viruses were evaluated, the rodent parvovirus assay detected only rodent parvovirus isolates, whereas the MVM and MPV/HaPV assays detected only the MVM or MPV/ HaPV isolates, respectively. Each assay detected the equivalent of 10 or fewer copies of target template, and all fluorogenic nuclease **PCR** assays exceeded the sensitivities associated with previously reported **PCR** assays and mouse antibody production testing. In addition, each fluorogenic nuclease **PCR** assay detected the targeted parvovirus DNA in tissues obtained from mice experimentally infected with MVM or MPV. Results of these studies indicate that fluorogenic nuclease **PCR** assays provide a potentially high-throughput, **PCR**-based method to detect rodent parvoviruses in infected mice and contaminated biological materials.

=> d l7 ibib abs 31-43

L7 ANSWER 31 OF 43 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2001258775 EMBASE
TITLE: Improved autoprocessing efficiency of mutant subtilisins E with altered specificity by engineering of the pro-region.
AUTHOR: Takahashi M.; Hasuura Y.; Nakamori S.; Takagi H.
CORPORATE SOURCE: M. Takahashi, Department of Bioscience, Fukui Prefectural University, Fukui 910-1195, Japan. mastak@fpu.ac.jp
SOURCE: Journal of Biochemistry, (2001) 130/1 (99-106).
Refs: 33

ISSN: 0021-924X CODEN: JOBIAO
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Modification of substrate specificity of an autoprocessing enzyme is accompanied by a risk of significant failure of self-cleavage of the pro-region essential for activation. Therefore, to **enhance processing**, we engineered the pro-region of mutant subtilisins E of Bacillus subtilis with altered substrate specificity. A high-activity mutant subtilisin E with Ile31Leu replacement (I31L) as well as the wild-type enzyme show poor recognition of acid residues as the P1 substrate. To increase the P1 substrate preference for acid residues, Glu156Gln and Gly166Lys/Arg substitutions were introduced into the I31L gene based upon a report on subtilisin BPN' [Wells et al. (1987) Proc. Natl. Acad. Sci. USA 84, 1219-12231. The apparent P1 specificity of four mutants (E156Q/G166K, E156Q/G166R, G166K, and G166R) was extended to acid residues, but the halo-forming activity of Escherichia coli expressing the mutant genes on skim milk-containing plates was significantly decreased due to the lower autoprocessing efficiency. A marked increase in active enzyme production occurred when Tyr(-1) in the pro-region of these mutants was then replaced by Asp or Glu. Five mutants with Glu(-2)Ala/Val/Gly or Tyr(-1)Cys/Ser substitution showing enhanced halo-forming activity were further isolated by **PCR** random **mutagenesis** in the pro-region of the E156Q/G166K mutant. These results indicated that introduction of an optimum arrangement at the cleavage site in the pro-region is an effective method for obtaining a higher yield of active enzymes.

L7 ANSWER 32 OF 43 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2001000852 EMBASE
TITLE: CpG DNA induces maturation of dendritic cells with distinct effects on nascent and recycling MHC-II antigen-processing mechanisms.
AUTHOR: Askew D.; Chu R.S.; Krieg A.M.; Harding C.V.
CORPORATE SOURCE: Dr. C.V. Harding, Department of Pathology, Case Western Reserve Univ. BRB925, 10900 Euclid Avenue, Cleveland, OH 44106, United States. cvh3@po.cwru.edu
SOURCE: Journal of Immunology, (15 Dec 2000) 165/12 (6889-6895). Refs: 46
ISSN: 0022-1767 CODEN: JOIMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Murine bone marrow cultured with GM-CSF produced dendritic cells (DCs) expressing MHC class II (MHC-II) but little CD40, CD80, or CD86. Oligodeoxynucleotides (ODN) containing CpG motifs enhanced DC maturation, increased MHC-II expression, and induced high levels of CD40, CD80, and CD86. When added with Ag to DCs for 24 h, CpG ODN **enhanced** Ag **processing**, and the half-life of peptide:MHC-II complexes was increased. However, Ag **processing** was only transiently **enhanced**, and exposure of DCs to CpG ODN for 48 h blocked processing of hen egg lysozyme (HEL) to HEL(48-61):I-A(k) complexes. Processing of this epitope required newly synthesized MHC-II and was blocked by brefeldin A (BFA), suggesting that reduced MHC-II synthesis could explain decreased processing. Real-time quantitative **PCR** confirmed that CpG ODN decreased I-A(βk) mRNA in DCs. In contrast, RNase(42-56):I-A(k) complexes were generated via a different processing mechanism that involved recycling MHC-II and was partially resistant to

BFA. Processing of RNase(42-56):I-A(k) persisted, although at reduced levels, after CpG-induced maturation of DCs, and this residual processing by mature DCs was completely resistant to BFA. Changes in endocytosis, which was transiently enhanced and subsequently suppressed by CpG ODN, may affect Ag processing by both nascent and recycling MHC-II mechanisms. In summary, CpG ODN induce DC maturation, transiently increase Ag processing, and increase the half-life of peptide-MHC-II complexes to sustain subsequent presentation. Processing mechanisms that require nascent MHC-II are subsequently lost, but those that use recycling MHC-II persist even in fully mature DCs.

L7 ANSWER 33 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:891926 CAPLUS
DOCUMENT NUMBER: 135:71786
TITLE: Plasmid-enhanced strategy for **PCR**-mediated **mutagenesis** with difficult DNA templates
AUTHOR(S): Abeyrathne, Priyanka D.; Nazar, Ross N.
CORPORATE SOURCE: University of Guelph, Guelph, ON, N1G 2W1, Can.
SOURCE: BioTechniques (2000), 29(6), 1172, 1174, 1176
CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Mutagenesis** by the **PCR** megaprimer method with DNA **sequences** containing repeating elements or encoding extensively paired RNA mols. can be highly recalcitrant to homogeneous amplification, resulting in very heterogeneous populations of products. The recombinations can be avoided or minimized by a simplified alternative approach in which the need for strand purification is eliminated entirely. With plasmid-enhanced **PCR**-mediated **mutagenesis**, the mutated DNA is amplified as two halves of the final **sequence** and joined by blunt ligation. For deletions, the targeted **sequence** is simply omitted, and, for substitutions, a mutant **sequence** is incorporated in one of the primers. The efficiency and orientation of the blunt ligation **process** is **enhanced** or controlled by **sequence**-specific overlapping interactions with the plasmid. To ensure a ligation at the blunt junction, the internal primers are 5'-phosphorylated before use and, in addition, the external primers must preced or include two unique restriction sites.
REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 34 OF 43 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 2001129186 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10984170
TITLE: A comparative study of the structure of the rDNA intergenic spacer of Lens culinaris Medik., and other legume species.
AUTHOR: Fernandez M; Polanco C; Ruiz M L; Perez de la Vega M
CORPORATE SOURCE: Departamento de Genetica, Facultad de Biologia, Universidad de Leon, Spain.
SOURCE: Genome / National Research Council Canada = Genome / Conseil national de recherches Canada, (2000 Aug) 43 (4) 597-603.
Journal code: 8704544. ISSN: 0831-2796.
PUB. COUNTRY: Canada
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ245998
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301
AB As part of a project on lentil molecular genetics, the **sequence** of the 18S-25S ribosomal RNA gene intergenic spacer (IGS) of Lens

culinaris Medik. was determined. DNA was **cloned** after **polymerase** chain reaction (**PCR**) amplification. The spacer of 2939 bp was composed of nonrepetitive **sequences** and four tandem arrays of repeated **sequences**, named A to D. C and D arrays were formed by the repetition of very short consensus **sequences.** Similarity was found between lentil and other legume species, in particular those of the Vicieae tribe. A transcription initiation site, putative sites of termination and **processing**, and promoter-**enhancer sequences** were detected by computer-aided searches. These sites resemble motifs conserved in the IGS **sequences** of other plant species. The conservation of motifs in the otherwise highly variable plant IGS **sequences** points to the relevance of these motifs as functional **sequences.**

L7 ANSWER 35 OF 43 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 2000266073 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10804149
TITLE: Detection of norwalk-like virus in shellfish implicated in illness.
AUTHOR: Shieh Y; Monroe S S; Fankhauser R L; Langlois G W; Burkhardt W 3rd; Baric R S
CORPORATE SOURCE: USFDA Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528, USA.. yshieh@cfsan.fda.gov
SOURCE: Journal of infectious diseases, (2000 May) 181 Suppl 2 S360-6. Ref: 47
Journal code: 0413675. ISSN: 0022-1899.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000719

AB In the 1990s, Norwalk-like viruses (NLVs) were identified in patient specimens as the primary pathogen associated with shellfish-borne gastroenteritis in the United States. Identification of these viruses from implicated shellfish has been difficult due to inefficient recovery of viruses, natural **polymerase** chain reaction (**PCR**) inhibitors in shellfish, and low virus contamination. Recent improvements to the method of detecting NLVs in shellfish include **enhanced processing** of virus and shellfish samples, application of nested **PCR** and nucleotide **sequencing**, and increased knowledge of NLV genetic diversity. Using a newly developed and sensitive method, an NLV G2 strain was identified in 2 oyster samples implicated in a 1998 California outbreak involving 171 cases. NLV capsid primers demonstrated a greater specificity of **PCR** detection than did **polymerase** primers. The 175-base viral capsid nucleotide **sequences** derived from oysters were 100% identical to those derived from a patient stool sample. This finding supports the epidemiologic associations indicating that contaminated shellfish serve as the vehicle for NLV transmission.

L7 ANSWER 36 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:779373 CAPLUS
DOCUMENT NUMBER: 132:10499
TITLE: Mutation analysis via mass spectrometry
PATENT ASSIGNEE(S): Bruker Daltonik G.m.b.H., Germany
SOURCE: Ger. Offen., 16 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19824280	A1	19991202	DE 1998-19824280	19980529
US 2002162622	A1	20021107	US 1999-321005	19990527
US 6503710	B2	20030107		
GB 2339279	A1	20000119	GB 1999-12584	19990528
GB 2339279	B2	20031119		

PRIORITY APPLN. INFO.: DE 1998-19824280 A 19980529

AB A method for mass spectrometric detection of substitution, deletion, and insertion mutations in DNA is disclosed. The method comprises **PCR** amplification of the target, mutation-containing DNA **sequence**; addition of a special set of modified dNTPs to support a limited enzymic elongation of an added primer; **polymerase**-mediated elongation of the primer; degradation and removal of the original primer, leaving only the enzymically added nucleotides and, optionally, addnl. chemical modification of the product; and, mass spectrometric anal. of the product. The special set of modified dNTPs consists of dNTPs which restrict the extent of the reaction (e.g., inclusion of ddNTP and exclusion of one of the dNTPs) and differentiate the wild-type from the mutant target. The dNTPs addnl. are initially modified, or modified subsequent to the elongation so that the DNA chain is stabilized during the ionization process, adduct formation is minimized, the ionization **process** is **enhanced**, and/or the mass of the DNA chain is altered. The primer may be removed with a phosphatase. The modified dNTPs may consist of α-thio-dNTPs and the oligonucleotide added to the primer may be modified by methylation of the thio groups.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 37 OF 43 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:78983 BIOSIS

DOCUMENT NUMBER: PREV200000078983

TITLE: Correction of aberrant splicing of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by antisense oligonucleotides.

AUTHOR(S): Friedman, Kenneth J.; Kole, Jolanta; Cohn, Jonathan A.; Knowles, Michael R.; Silverman, Lawrence M.; Kole, Ryszard [Reprint author]

CORPORATE SOURCE: Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, USA

SOURCE: Journal of Biological Chemistry, (Dec. 17, 1999) Vol. 274, No. 51, pp. 36193-36199. print.
CODEN: JBCHA3. ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 23 Feb 2000
Last Updated on STN: 3 Jan 2002

AB The CFTR splicing mutation 3849 + 10 kb C fwdarw T creates a novel donor site 10 kilobases (kb) into intron 19 of the gene and is one of the more common splicing mutations that causes cystic fibrosis (CF). It has an elevated prevalence among patients with atypically mild disease and normal sweat electrolytes and is especially prominent in Ashkenazi Jews. This class of splicing mutations, reported in several genes, involves novel splice sites activated deep within introns while leaving wild-type splice elements intact. CFTR cDNA constructs that modeled the 3849 + 10 kb C fwdarw T mutation were expressed in 3T3 mouse fibroblasts and in CFT1 human tracheal and C127 mouse mammary epithelial cells. In all three cell types, aberrant splicing of CFTR pre-mRNA was comparable to that reported in vivo in CF patients. Treatment of the cells with 2'-O-methyl phosphorothioate oligoribonucleotides antisense toward the aberrant donor and acceptor splice sites or to the retained exon-like **sequence**, disfavored aberrant splicing and **enhanced** normal **processing** of CFTR pre-mRNA. This antisense-mediated correction

of splicing was dose- and **sequence**-dependent and was accompanied by increased production of CFTR protein that was appropriately glycosylated. Antisense-mediated correction of splicing in a mutation-specific context represents a potential gene therapy modality with applicability to many inherited disorders.

L7 ANSWER 38 OF 43 MEDLINE on STN
ACCESSION NUMBER: 1999063697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9847186
TITLE: New features of the Blocks Database servers.
AUTHOR: Henikoff J G; Henikoff S; Pietrokovski S
CORPORATE SOURCE: Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109-1024, USA.
CONTRACT NUMBER: GM29009 (NIGMS)
SOURCE: Nucleic acids research, (1999 Jan 1) 27 (1) 226-8. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990326
Last Updated on STN: 19990326
Entered Medline: 19990316

AB Blocks are ungapped multiple **sequence** alignments representing conserved protein regions, and the Blocks Database consists of blocks from documented protein families. World Wide Web (http://www.blocks.fhcrc.org) and Email (blocks@blocks.fhcrc.org) servers provide tools for homology searching and for analyzing protein family relationships. New **enhancements** include a multiple alignment **processor** that extends the use of these tools to imported multiple alignments of families not present in the database and a **PCR** primer designer that implements a new strategy for gene isolation.

L7 ANSWER 39 OF 43 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1998:660335 SCISEARCH
THE GENUINE ARTICLE: 113KY
TITLE: Genetic purity and testing technologies for seed quality: a company perspective
AUTHOR: Smith J S C (Reprint); Register J C
CORPORATE SOURCE: PIONEER HI BRED INT INC, RES & PROD DEV, POB 1004, JOHNSTON, IA 50131 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: SEED SCIENCE RESEARCH, (JUN 1998) Vol. 8, No. 2, pp. 285-293.
Publisher: C A B INTERNATIONAL, C/O PUBLISHING DIVISION, WALLINGFORD OX10 8DE, OXON, ENGLAND.
ISSN: 0960-2585.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: English
REFERENCE COUNT: 31
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A high level of genetic purity in crop varieties must be achieved and maintained for agronomic performance as well as to encourage investment and innovation in plant breeding and to ensure that the improvements in productivity and quality imparted by breeders are delivered to the farmer and, ultimately, to the consumer. Traditionally, morphological comparisons have formed the basis for genetic purity evaluations. However, replicated field observations are time-consuming, expensive and unreliable. Morphology cannot provide information on the purity of specific genetic attributes that relate to grain quality or to pest or herbicide resistance bred into varieties. Biochemical assays, including isozymes, can distinguish varieties within several species. Isozymes have been routinely

used in checking seed-lot purity in maize (Zea mays L.) for the past 20 years. Newer DNA-based technologies such as restriction fragment length polymorphisms and more recently developed methods that use the **polymerase** chain reaction can allow even more discriminative and faster identification of varieties. However, none of the DNA methods have replaced biochemical methods for seed purity assays, other than in a relatively select group of crops with very high seed value, due to their high datapoint cost. It will require further miniaturization, automation and **enhanced** capabilities to **process** numerous samples simultaneously before newly developed methods supplant biochemical methods for routine usage in purity testing. New varieties that have major genes for herbicide or insect resistance incorporated within them require purity assays during product development and following seed production of the commercial variety. Immunological or DNA **sequence** assays can be developed and automated systems are required to process hundreds of thousands of individuals. Ultra-high, micro-array technologies and single-molecule detection systems are now under development. These technologies offer the promise that adequate distinction and high sample throughput will be combined. New methods may eclipse the capabilities of biochemical methodologies, thereby potentially raising genetic purity standards and enabling farmers and consumers better to utilize and benefit from increasingly productive Varieties that are bred from a more diverse base of genetic resources.

L7 ANSWER 40 OF 43 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1997-11237 BIOTECHDS
TITLE: Amplification of nucleic acids by **PCR**; **polymerase** chain reaction with increased efficiency by peptide nucleic acid annealing
AUTHOR: Demers D B
PATENT ASSIGNEE: Genet.IVF-Inst.Fairfax
LOCATION: Fairfax, VA, USA.
PATENT INFO: US 5656461 12 Aug 1997
APPLICATION INFO: US 1995-468658 6 Jun 1995
PRIORITY INFO: US 1995-468658 6 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-414587 [38]
AN 1997-11237 BIOTECHDS
AB A new **process** for **enhancing polymerase** chain reaction (**PCR**) amplification of target DNA over 600 bp long (or over 1 or 10 kb long) with at least 1 perfect or imperfect repeat, by reducing preferential amplification and anomalous product generation, involves treating the sample with at least 1 peptide nucleic acid (PNA) binding the target **sequence**, prior to amplification. At least 1 DNA primer is annealed to the target DNA, and is extended, with PNA displacement from the template during primer extension. A mixture of PNAs (20-50 monomers in length) may be used, and may form a duplex with part of the target DNA, with a melting temperature of 70-80 deg (preferably 70-78 deg). Preferably, the **PCR** denaturation temperature is 80-100 deg, the PNA binding temperature is 70-80 deg, and the primer binds at 40-80 deg. The PNA may be displaced at 60-80 deg. An improved method is claimed. The method may be used in amplification, **cloning** and in vitro **mutagenesis**, or in infectious or genetic disease diagnosis, and increases the size of template span which may be extended successfully in **PCR**. (22pp)

L7 ANSWER 41 OF 43 MEDLINE on STN
ACCESSION NUMBER: 96400450 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8806826
TITLE: Regulated demethylation of the myoD distal enhancer during skeletal myogenesis.
AUTHOR: Brunk B P; Goldhamer D J; Emerson C P Jr
CORPORATE SOURCE: Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia 19104-6058,

USA.
CONTRACT NUMBER: AR-42644 (NIAMS)
HD-07796 (NICHD)
SOURCE: Developmental biology, (1996 Aug 1) 177 (2) 490-503.
Journal code: 0372762. ISSN: 0012-1606.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-S82831
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961106
Last Updated on STN: 19980206
Entered Medline: 19961024

AB myoD is one of a family of four related basic helix-loop-helix transcription factors involved in the specification and differentiation of skeletal muscle. We previously identified a 258-bp distal enhancer that is sufficient for embryonic activation of myoD and is highly conserved between humans and mice. In this paper, we show using a modified bisulfite deamination/**PCR** amplification method that the distal myoD enhancer is completely unmethylated at all the CpG sites tested in myogenic cells and a subpopulation of somite cells. Conversely, the distal enhancer in nonmuscle cells and tissues is methylated to an average level of > 50% and we find no chromosomes in these tissues with a completely unmethylated enhancer. We present evidence that demethylation of the distal enhancer in somites of mouse embryos precedes myoD transcription, suggesting that demethylation of the distal **enhancer** is an active, regulated **process** that is essential for myoD activation. We also show by analysis of transgenic mice carrying a human distal enhancer/reporter construct in which the three enhancer CpG sites have been mutated that methylation of the distal enhancer is not required to prevent precocious or ectopic embryonic myoD expression. We propose that a subset of somite cells demethylate the distal enhancer in response to specific developmental signals, thus making the enhancer accessible and able to respond to subsequent signals to activate the myoD gene.

L7 ANSWER 42 OF 43 CANCERLIT on STN
ACCESSION NUMBER: 93373056 CANCERLIT
DOCUMENT NUMBER: 93373056 PubMed ID: 8364724
TITLE: Target regulation of neuronal differentiation in a temperature-sensitive cell line derived from medullary raphe.
AUTHOR: Whittemore S R; White L A
CORPORATE SOURCE: Miami Project, University of Miami School of Medicine, FL 33136.
CONTRACT NUMBER: NS26887 (NINDS)
SOURCE: BRAIN RESEARCH, (1993 Jun 25) 615 (1) 27-40.
Journal code: 0045503. ISSN: 0006-8993.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: MEDLINE; Priority Journals
OTHER SOURCE: MEDLINE 93373056
ENTRY MONTH: 199310
ENTRY DATE: Entered STN: 19941107
Last Updated on STN: 19941107

AB Following infection of dissociated embryonic day 13 rat medullary raphe cells with a retrovirus encoding the temperature-sensitive mutant of SV40 large T antigen, a **clonal** cell line, RN33B, was isolated by serial dilution. At 33 degrees C, RN33B cells divide with a doubling time of 48 h and show T antigen, vimentin, nestin, diffuse neuron-specific enolase, and low and medium molecular weight neurofilament immunoreactivities. RN33B cells are immortal, but not transformed, as they will not grow in soft agar. At non-permissive temperature (38.5 degrees

C), T antigen expression is markedly decreased and RN33B cells cease mitotic activity and differentiate with phase bright cell bodies and 'neuritic-like' **processes.** Differentiated RN33B cells express **enhanced** neuronal-specific protein expression but do not synthesize astrocytic or oligodendrocytic-specific proteins. Moreover, differentiated RN33B cells returned to 33 degrees C re-express T antigen, but do not de-differentiate or begin dividing. Co-culture with embryonic hippocampus and cerebral cortex, but not medullary raphe or spinal cord, resulted in significantly greater survival, more complex neuronal morphology, and enhanced expression of neuronal-specific antigens. Immunohistochemical and Northern blot analysis revealed high levels of low affinity NGF receptor protein and mRNA in differentiated RN33B cells. **PCR** analysis demonstrated the presence of trkB, but not trkA or trkC, mRNA in both undifferentiated and differentiated RN33B cells. These data suggest that the observed target regulation of RN33B cell neuronal differentiation in co-culture may be mediated by neurotrophin(s).

L7 ANSWER 43 OF 43 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:154972 CAPLUS
DOCUMENT NUMBER: 120:154972
TITLE: Cost-effective gene synthesis, multiple **mutagenesis** and gene fusion by **polymerase** chain reaction
AUTHOR(S): Majumder, Kumud
CORPORATE SOURCE: Int. Cent. Genet. Eng. Biotechnol., New Delhi, 110 067, India
SOURCE: Downstream Process. Biotechnol., Proc. Int. Semin. (1992), Meeting Date 1991, 198-215. Editor(s): Mukherjea, R. N. Tata McGraw-Hill: New Delhi, India. CODEN: 59PLAW
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with 27 refs. A novel method for the synthesis of synthetic genes involving stepwise elongation of **sequence** through the **polymerase** chain reaction (SES-**PCR**) was developed to **enhance** the ease of downstream **processing** of genetically engineered and expressed proteins. This cost-effective method allows the synthesis of a gene unencumbered by the kination (sic) and ligation steps. Further, site-directed **mutagenesis** and/or gene fusion by SES-**PCR** is not limied by the prior availability of the gene(s) in question. The potential of SES-**PCR** in gene synthesis, **mutagenesis** at multiple sites, and gene fusion was demonstrated using a chimeric gene encoding a fusion between the OmpA signal peptide and hirudin. The product gene was **cloned** and **sequenced** to show that the gene bears only the desired mutations. A nearly 100% efficiency of **mutagenesis** was achieved by the method.

=> d his

(FILE 'HOME' ENTERED AT 17:25:28 ON 08 APR 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 17:25:47 ON 08 APR 2004
L1 38 S POLYMERASE ENHANCER
L2 625 S (POLYMERASE AND (ENHANC? (4A) PROCESS?))
L3 0 S L2 AND DUP REM
L4 1 S L1 AND REPLICATION
L5 136 S L2 AND PCR
L6 68 S (L5 AND (CLON? OR SEQUENC? OR MUTAGENESIS))
L7 43 DUP REM L6 (25 DUPLICATES REMOVED)

=> end
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	119.36	119.57

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-4.85	-4.85

STN INTERNATIONAL LOGOFF AT 17:36:04 ON 08 APR 2004